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The effects of stress hormones on human memory

THE EFFECTS OF STRESS HORMONES ON HUMAN MEMORY

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Abstract

The experiments presented in this thesis were based on the evidence of previous research that suggests that the memory functions dependent on the integrity of the hippocampus and frontal lobes, namely declarative and working memory respectively, are sensitive to the effects of corticosteroids (stress hormones). The first experiment investigated the effects of acute changes of three different levels of cortisol (high vs. control vs. low) and time of day (am vs. pm) on working memory and the episodic and semantic components of declarative memory. This was carried out using a single-blind, mixed (3 x 2) design with three groups of young, healthy males (N = 20 per group). Whilst significant differences in salivary cortisol levels were observed, the results failed to demonstrate any significant differences in any aspect of memory performance as a function of corticosteroids. However, whilst the results also failed to demonstrate significant differences in either aspect of memory performance as a function of time of day, they did identify a significant positive relationship between morning cortisol levels in the control group and two measures of episodic memory in the morning; this suggests that, in the morning, these aspects of memory performance were facilitated by higher cortisol levels. They also identified a significant negative relationship between afternoon cortisol levels in the high cortisol group and one measure of semantic memory in the afternoon; this suggests that, in the afternoon, this aspect of memory performance was impaired by higher cortisol. The second experiment investigated the effects of acute changes in corticosteroids following activation of the different corticosteroid receptors on working memory and the episodic and semantic components of declarative memory. This was carried out using a repeated measures design with nine patients with Addison's disease. The results suggest that, whilst significant effects were not identified across all memory tasks, activation of the mineralocorticoids appears essential during sensory storage (i.e., encoding) whereas activation of the glucorticoids appears essential during memory consolidation and retrieval. This supports previous research carried out in rats (Oitzl & De Kloet, 1992). The results also suggest that balanced activation of the mineralocorticoids and glucocorticoids is necessary for optimal memory function. The contributions made by both experiments are discussed.

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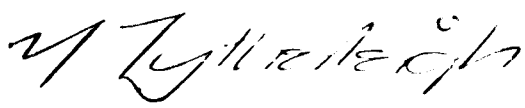
Author's Declaration

The work contained in this thesis is that of the author and was carried out in accordance with the regulations of the University of Bristol. Any views expressed in this thesis are those of the author and in no way represent those of the University of Bristol. This thesis has not been presented to any other University for examination either in the United Kingdom or overseas.

Signed :  Date : November 2002

Contents

Table of Figures

Table of Tables

1. Introduction and Background to the problem

1.1 The corticosteroid-response to stress

Stress has been defined as both a 'physiological and psychological construct'. It is the second highest cause of absenteeism in the UK, with 30% of sick leave related to workplace stress (CBI, 1997) and costing UK employers around £12 billion every year (HSE, 1998). Consequently, investigations into the physiological and psychological aspects of stress form a very significant area of research.

Stress is the body's response to the demands placed upon it, and it is only by understanding these demands and the effects produced, that individuals can learn to recognize their own stress responses and ways to counteract them. However, the effects of stress are complex. Indeed, the effects produced can be physiological, emotional, cognitive and/or behavioural. Consequently, the way in which the body deals with stress can require adaptation on all four levels.

The subject area of this thesis concerns the cognitive effects of stress and, more specifically, the effects of corticosteroids (stress hormones) on memory performance. Previous research has identified several effects of corticosteroids on a variety of memory functions. In particular, it is evident that both endogenous- and exogenous-based changes in corticosteroids (i.e., those produced naturally versus those produced synthetically using steroids) are associated with deficits in both memory and attention (Lupien, Lecours, Lussier et al., 1994; Lupien, Gaudrea, Tchiteya et al., 1997). The effects on memory are also similar to those produced by ageing (Seeman, McEwen, Singer, Albert & Rowe, 1997) and this suggests that by

increasing our understanding of the effects of corticosteroids on memory this may also increase our understanding of the ageing process itself.

The effects of endogenous versus exogenous corticosteroids on memory, although different in significant aspects, are also very similar. Detrimental effects on memory have been identified following stress-induced increases in corticosteroids (Kirschbaum, Wolf, May, Wippich & Hellhammer, 1996) and following the administration of steroids (e.g., Keenan, Jacobson & Soleymani, 1995). As many prescriptions per year are written for therapeutic doses of synthetic steroids to treat many disorders (e.g., asthma, arthritis), the detrimental effects of corticosteroids may have clinical implications for the use of steroid-therapy.

However, although the effects of corticosteroids have been shown to impact on memory performance, the effects produced are not always negative. Indeed, the Yerkes & Dodson model of arousal has been applied to the corticosteroid-related effects on performance (Yerkes & Dodson, 1908). This suggests that there is an inverted U-shaped relationship between corticosteroids and memory, whereby levels of corticosteroids which are too high or too low can impair memory performance, but optimum levels of corticosteroids can facilitate it (Lupien et al., 1997). Indeed, it is this inverted U-shaped relationship between corticosteroids and memory that explains the magnitude and direction of the effects produced.

Notwithstanding this, however, there are clearly several other factors that have been shown to modify the effects of corticosteroids on memory. Some of these have been clearly identified (e.g., the effects of dose, duration of treatment, timing of treatment relative to learning, specific type of task used, and additional effects of age and gender); some, however, still remain unclear. These include: the additional effects of time of day; the difference between acute versus chronic levels of

corticosteroids; and the difference in effects produced via activation of the two different corticosteroid receptors. In addition to their effects on memory, these factors may also have implications for the effects on ageing and other important aspects of health (e.g., immunosuppression). The purpose of this thesis, therefore, is to describe what previous research has clearly identified regarding the effects of corticosteroids on memory and then report the details of two studies that were carried out to explore those factors which still remain unclear. The structure of this thesis, therefore, is as follows:-

- Chapter 1 provides a general introduction to the corticosteroid hormones and the part they play in the cortisol-response to stress. This includes a review of the previous research and a summary of the issues that remain unclear.

- Chapter 2 describes the methods and results of Experiment 1 - the dose-range study - which was carried out to identify the levels of medication to be administered to participants in Experiment 2. This chapter also describes the reason why medication was used to manipulate cortisol levels (i.e., to control individual differences in cortisol-response).

- Chapter 3 describes the methods and results of Experiment 2 – a study using healthy, young males – which was carried out to identify the effects of three different acute changes in cortisol levels on memory together with the additional effects of time of day.

- Chapter 4 describes the methods and results of Experiment 3 – a study using patients with Addison's disease – which was carried out to identify the effects on memory produced following differential activation of the two corticosteroid receptors (i.e., the mineralocorticoid receptors [MRs] versus the glucocorticoid receptors [GRs]). Although this condition has been previously investigated in rats and chickens (e.g., Oitzl & de Kloet, 1992; Sandi & Rose, 1994), prior to this study it had not been investigated humans.

- Chapter 5 also describes the results of Experiments 2 and 3, but more specifically the results which were produced using the same item-recognition task used by Lupien, Gillin & Hauger (1999). Whilst these results do not focus on the effects of corticosteroids on memory, they identify the type of cognitive search strategy used by participants to perform the task and how this plays a significant part in the interpretation of results.

- Chapter 6 - the final chapter - provides an overall summary and global conclusion to the thesis. This includes a re-evaluation of the evidence pertaining to the effects of corticosteroids on memory, with some suggestions for future research.

Before defining and describing the role of corticosteroids, however, there are certain terms and aspects of memory that the reader needs to be aware of in order to understand the corticosteroid-memory association. These are described as follows:

1.1.1 Corticosteroids : definition and function

As defined earlier, corticosteroids are steroid hormones secreted by the adrenal cortex that 'coordinate, together with other components of the stress system, the organism's ability to cope with stress' (De Kloet, Oitzl & Joels, 1999; p.422). The principal corticosteroid in humans is cortisol. When a stress response is evoked, the sympathetic nervous system (SNS) drives arousal and prepares the body for 'fight or flight'. This is when cortisol, along with the other SNS hormones such as glucagon, adrenaline and noradrenaline, is released. Of all the stress hormones, however, it is cortisol that remains elevated in the body for the longest period after stress. Consequently, it is cortisol which can have the most important long-term effects on behaviour and health (McEwen, 1998; Munck, Guyre & Holbrook, 1984).

Under basal conditions cortisol is released with a diurnal rhythm, with maximal plasma levels being achieved early in the morning followed by levels that gradually fall throughout the day to low levels at night. This rhythm is under hypothalamic regulation by the supra chiasmatic nucleus. As described above, in addition to this endogenous rhythm cortisol can also be released in response to stressors in a reactive manner to help the individual cope with, adapt to, or recover from the stressful situation.

Cortisol can be released during periods of both acute (Al Absi et al., 1997) and chronic (Vedhara, Cox, Wilcock et al., 1999) stress. Indeed, cortisol levels are widely regarded as an objective index of changes in psychological stress (Kirschbaum, Prussner, et al.,1995). Acute periods of controllable stress can be beneficial. For example, in the short-term the release of cortisol can increase energy levels, whereas in the longer term, it can

enhance the body's resilience to future stress (Epel, McEwen & Ockowics, 1998). Alternatively, however, the effects of chronically raised levels of cortisol can be detrimental. For example, overexposure to cortisol can damage many systems (McEwen, 1998; McEwen & Stellar, 1993; Munck et al., 1984) including fat metabolism, glucose production, inflammatory and vascular responses, and the functioning of the central nervous system (CNS) and immune systems (Stone, Schwartz, Smyth et al., 2001). High levels of cortisol can also impair the negative feedback suppression of further cortisol secretion (i.e., its reactive response), which results in a poorer shut-off response and slower cortisol recovery (Sapolsky, Romero & Munck, 2000). In addition, increased levels of cortisol can have detrimental effects on memory performance.

1.2. Possible effects of corticosteroids on memory

Cortisol-related memory impairments have been identified in humans in response to both endogenously and exogenously induced increases in cortisol (i.e., hypercortisolism). The common finding is that chronic elevations impair declarative/explicit memory (defined later), leaving the non-hippocampal forms (i.e., procedural/implicit memory) unimpaired (Lupien et al., 1994, 1997; Newcomer, Craft, Hershey, Askins & Bardgett, 1994;Seeman et al., 1997). Moreover, more recent evidence suggests that working memory (which relies on the integrity of the frontal lobes) may be more sensitive to acute changes in cortisol levels than declarative memory (Lupien et al., 1999).

Before reviewing the effects of cortisol on declarative and working memory, however, the features of long-term memory and the formulation of long-term memories need to be defined. This will help explain the selective effects of cortisol on memory and why it is the memory functions dependent on the integrity of the hippocampus and frontal lobes that appear to be the most vulnerable.

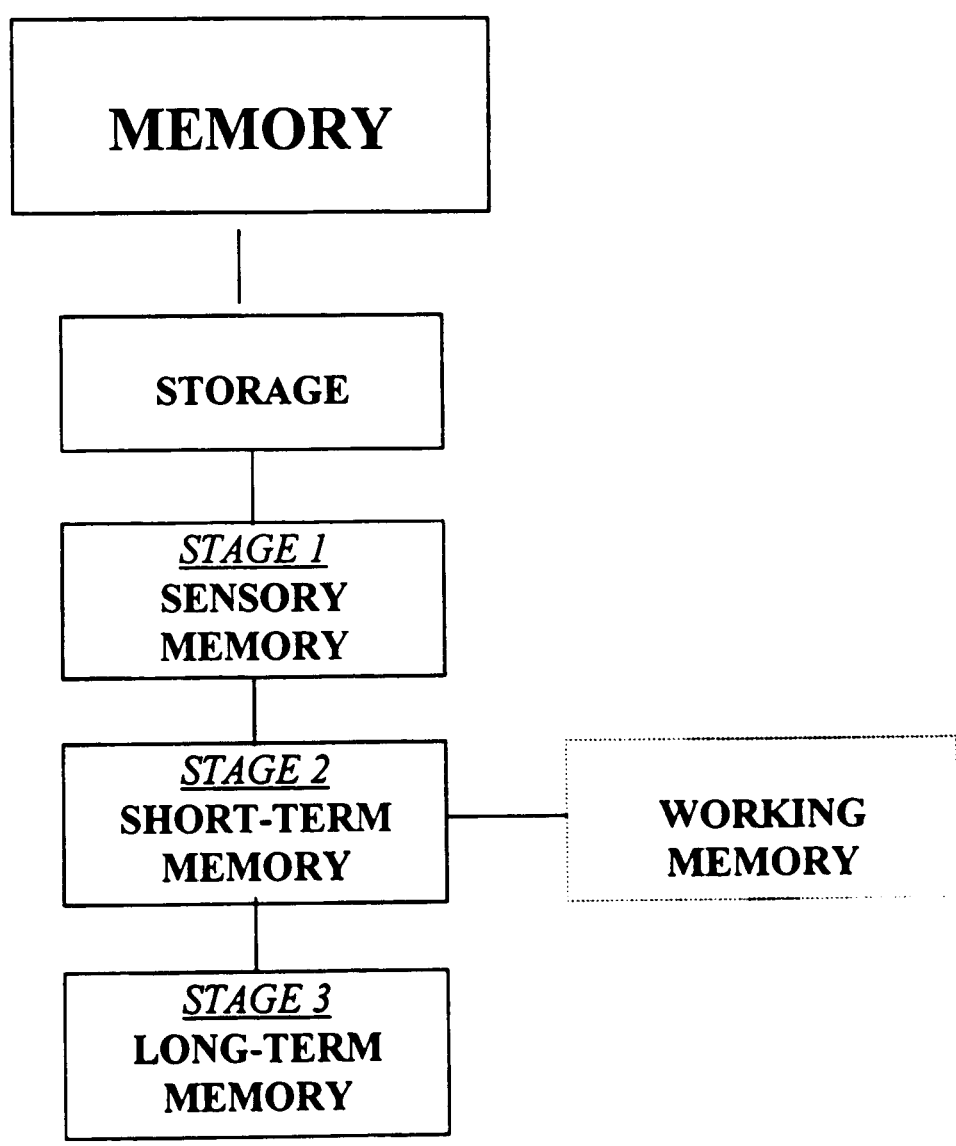
1.2.1 Features of Long-term Memory

The storage of memories in the long-term memory process comprises three stages. These are illustrated in Figure 1. During the first stage, or sensory memory, our senses register/encode a 'literal', though not complete, copy of the stimulus to which we are exposed (Gross, 1996). If successfully encoded, this information is then passed during the second stage into short-term storage where, unless repeated or rehearsed, it will remain for approximately 15-30 seconds. This short-term store is often referred to as short-term or working memory (Atkinson & Shiffrin, 1968; Baddeley & Hitch, 1974). The transfer of information from working memory to long-term memory takes place during the final stage of the process. There is no evident limit to the amount of information that can be stored in long-term memory and it is this system which allows us to consolidate old information with new. The transfer of information into long-term memory can, however, breakdown at any one of the three storage stages (i.e., during acquisition/encoding, consolidation and/or retrieval). The effects of cortisol can occur at any one of these.

Some studies have clearly identified the stage during the long-term memory process at which the effects of cortisol occur. For example, detrimental effects have been reported during: acquisition (Lupien et al.,

1999); consolidation (Lupien et al., 1999; Lupien, Lecours, Schwartz et al., 1995); and retrieval (De Quervain, Roozendaal, Nitsch, McGaugh & Hock, 2000). Indeed, the common finding is that chronic elevations of cortisol impair the retrieval phase of declarative memory, whereas working memory and the acquisition and consolidation of information appears more sensitive to acute changes in cortisol levels (Lupien et al., 1999). As cortisol can have multiple and often conflicting effects on memory function, it is crucial to be able to dissociate the effects on the different memory phases in order to interpret the effects on memory correctly (Lupien et al., 1997).

Figure 1 : A summary of the three stages of memory storage and different forms of long-term memory



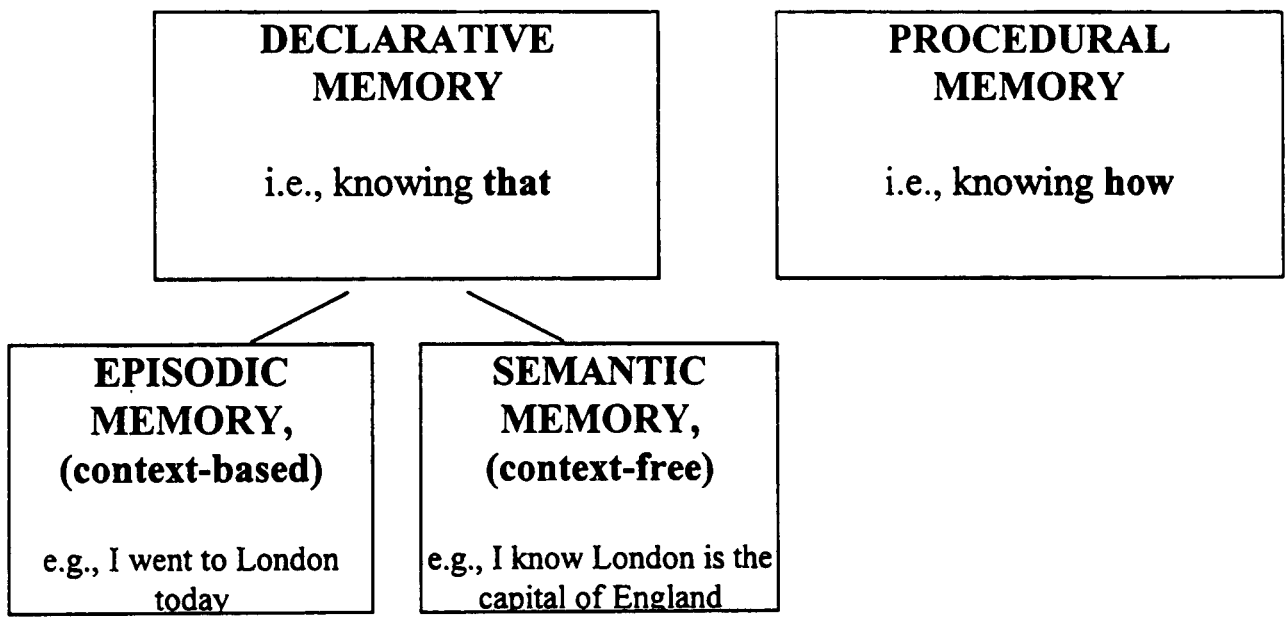
A task designed to measure long-term declarative memory can pick up deficits during any of the three storage stages. However, impaired long-term memory performance can be a result of incorrect encoding brought about by an overload of working memory (i.e., low free recall and low recognition performance). Alternatively, it can be a result of impairment at the level of recall caused by an impairment in declarative memory (i.e., low free recall but normal recognition). Consequently, it has been suggested that the best way to test whether cortisol impairs encoding and/or declarative memory is to use a recognition task in addition to a free recall task (Lupien et al., 1999).

There is one potential problem, however, with this interpretation of long-term memory and the effects of cortisol upon it. Whilst all items appear to pass through short-term/working memory, with working memory acting as the system used for temporarily holding and manipulating information (Baddeley, 1990), there is evidence to suggest that the effects of treatment used on rats to alter long-term memory (using receptor agonists and antagonists) show that the short-term and long-term memory systems are essentially separate mechanisms. Indeed recent animal data have indicated that such treatments as the serotonin 1A receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (DPAT), when given into the entorhinal cortex, can affect short-term memory without necessarily affecting later long-term memory (Izquierdo et al., 1998). In this particular case, short-term memory was enhanced and long-term memory was blocked. It is important to note, however, that at the time of writing this thesis the results of this study have not been replicated and, furthermore, this same effect has not been reported in humans.

1.2.2 *Definition/conceptualisation of declarative memory*

Figure 2 shows the features of long-term memory. As illustrated, declarative memory is the system that coincides with 'knowing that' (Ryle, 1949) and refers to the 'conscious or voluntary recollection of previous information' (Lupien & McEwen, 1997). For example, we know that London is the capital of England. In contrast, procedural memory coincides with 'knowing how' and how our previous experiences affect our recollection of previous information without priming (Lupien & McEwen, 1997).

Figure 2 : Features of Long-term Memory



As shown, there are two components to declarative memory. These comprise episodic memory and semantic memory (Squire, 1987; Tulving, 1983; Tulving, Hayman & McDonald, 1991). Episodic memory is context-based and refers to the storage of specific events that occurred in a particular place at a particular time. In contrast, semantic memory is context-free and provides a 'store of general, factual knowledge about the world, including

concepts, rules and language' (Gross, 1996). For example, episodic memory is remembering what you had for dinner last night, whereas semantic memory is knowing what the word 'dinner' means, or recognising the potatoes on your plate and knowing that they are edible. However, although episodic memory and semantic memory are generally seen as working alongside each other, episodic memory relies on temporal and spatial contextual cues for the retrieval of information, whereas semantic memory does not (Tulving, 1983). In line with previous research, the declarative memory functions being investigated in this thesis comprise both the episodic and semantic components.

1.2.3. A 'unitary' versus 'dissociated' theory of declarative memory

In addition to the two components of declarative memory, there are also two main perspectives on how the episodic and semantic components operate within declarative memory. According to the 'unitary' perspective, declarative memory is the memory for facts and events (Cohen, Poldrack & Eichenbaum, 1997; Squire & Knowlton, 1995; Squire & Zola, 1996). This perspective maintains that all items must pass through episodic memory before reaching semantic memory and, consequently, any impairment in episodic memory can result in an equivalent level of impairment in semantic memory. A more recent study, however, suggests that the episodic and semantic components of declarative memory are partly dissociated (Vargha-Khadem et al., 1997).

Vargha-Khadem et al. investigated the episodic and semantic memory functions of three young patients who each suffered severe hippocampal damage shortly after birth. Each patient reported severe difficulty in

remembering ongoing experiences (i.e., episodic memory), as expected. However, they also presented near-normal intellectual development (i.e., semantic memory); this was not expected. Consequently, Vargha-Khadem et al. interpreted this as showing that episodic memory is not critical for the formation of semantic memory. Furthermore, as damage to the hippocampus had resulted in deficits in learning but not remembering, they also suggested that only the episodic component of declarative memory is fully dependent on the hippocampus. According to the dissociated perspective, therefore, any impairment in episodic memory may not result in an equivalent, or indeed any, impairment in semantic memory. In addition, if semantic memory is not fully dependent on the hippocampus it could be predicted that any impairment to semantic memory would be much less, if at all, than any found in episodic memory.

Although some studies investigating the effects of cortisol on declarative memory have differentiated between the episodic and semantic components, this has not always been defined. Consequently, to investigate whether both aspects of declarative memory are affected by cortisol, or whether the episodic and semantic components are partly dissociable, independent tests of episodic and semantic memory need to be used. If deficits are then identified in episodic memory performance only, with semantic memory performance remaining unimpaired, this will lend support to a partly dissociable theory for declarative memory. The same effects may also give an indication as to whether semantic memory is dependent on the integrity of the hippocampus and if so, to what degree.

1.2.4. *Corticosteroid receptors and their relationship with declarative and working memory*

One explanation for the selective effects of cortisol on memory has been attributed to the organisation of memory and the abundance of corticosteroid receptors in specific areas of the brain. There are two types of corticosteroid receptors and these comprise the Type I mineralocorticoid receptors (MRs) and the Type II glucocorticoid receptors (GRs). Cortisol can bind to both types of receptors. Whereas GRs are widely distributed within the CNS, the distribution of MRs is much more limited. Indeed, the highest expression of corticosteroid receptors is found in the hippocampus, the structure involved in learning and memory (De Kloet, Vreugdenhil, Oitzl & Joels, 1998). As mentioned previously, the hippocampus is also an essential component of declarative memory (McEwen, 1997). Whilst long-term memory is not dependent on this structure alone, it is particularly dependent on the hippocampus when new memories are formed (Keenan & Kuhn, 1999). This helps explain why the functions dependent on the integrity of the hippocampus (i.e., declarative memory) are highly sensitive to elevations in cortisol.

Although the highest concentration of corticosteroid receptors is located in the hippocampus, however, this is not the only target for cortisol (Jacobson & Sapolsky, 1991; Sapolsky, 1992). A high concentration of corticosteroid receptors is also found in the prefrontal cortex. Moreover, the prefrontal cortex is also a significant target for the negative-feedback actions of circulating corticosteroids (Moghaddam, Bolinao, Stein-Behrens & Sapolsky, 1994). Research using brain scanning techniques has shown that it is the neurons located in the prefrontal cortex which are activated during

encoding (Smith, Jonides, Marshuetz & Koeppe, 1998;Ungerleider, Courtney & Haxby, 1998). Consequently, as the prefrontal cortex is also rich in corticosteroid receptors and it is the prefrontal cortex which holds information during short-term use (Goldman-Rakic, 1996), this suggests that working memory (which is dependent on the integrity of the frontal lobes) may also be sensitive to changes in cortisol. Indeed, a more recent study suggests that working memory may be more sensitive to acute changes in cortisol levels than declarative memory as 'a consequence of their specific effects on the attentional-dependent working memory system' (Lupien et al., 1999).

1.2.5. Mineralocorticoid vs. glucocorticoid receptors

As described previously, cortisol binds to both the MRs and GRs. However, the affinity of cortisol to the MRs is ten-fold higher than that of the GRs (De Kloet, 1991). Consequently, this means that low basal levels of cortisol activate the MRs. Indeed, under basal conditions, the MRs are 90% occupied whilst the GRs are only 50% occupied. Alternatively, the GRs are only activated when cortisol levels are high (e.g., during stress; Reul & De Kloet, 1985). However, although activation of the GRs appears to be a pre-requisite for the long-term storage of information (De Quervain, Roozendaal & McGauch, 1998), enhanced occupation can be detrimental. This is because activation of the MRs increases long term potentiation (LTP) – the phenomenon of increasing the responsiveness of neurons – which is dampened down by greater activation of the GRs (Kerr, Campbell, S-Y, & Landfield. 1989). A further increase in cortisol levels also results in additional occupancy of the GRs, which then impacts the negative feedback action and prevents

further downstream cortisol release (De Kloet & Reul, 1987); this has been referred to as 'induced long-term depression' (Pavlides, Kimura, Margarinos & McEwen, 1994). Taken together, therefore, it has been suggested that the behavioural deficits associated with the elevation of corticosteroids in humans and animals may be explained by increased activation of the GRs.

It is important to emphasize at this point, however, that it is not the increase in levels of cortisol per se. which can have the detrimental effects on memory. Rather, it is the influence that these increased levels can have on the information-processing systems (De Kloet et al., 1999). It is also important to emphasise that, as a result of the negative feedback actions of circulating cortisol levels, during higher levels of stress increased activation of the GRs occurs to dampen down the effects of stress.

Previous research in non-primates suggests that activation of the MRs and GRs affect different aspects of information processing (De Kloet et al., 1999). Studies carried out using selective MR and GR agonists and antagonists in rats, administered at different stages of information processing, suggest that activation of the MRs appears to be essential for interpreting and selecting new information. In contrast, activation of the GRs (in addition to the already activated MRs) appears to be essential for 'optimal memory' (i.e., remembering). According to this view, therefore, a deficiency or inhibition of the MRs would impair selective attention and sensory integration, making it difficult for an individual to discriminate between relevant and irrelevant cues. This, in turn, affects the process of memory acquisition and impacts on the other 'downstream' aspects of consolidation and retrieval. As a result, global deficits in learning and memory can occur. In contrast, a deficiency of the

GRs only affects the consolidation and retrieval aspects of memory. Rather, individuals may learn new information and retain past information for a limited period of time, however, they would be very susceptible to forgetting and interference. A summary of the differences between the Type I and Type II corticosteroid receptors is presented in Table I.

Table I : Summary of the differences between the Type I and Type II corticosteroid receptors

Receptor Type	
Type I – MRs	**Type II – GRs**
Activated by low basal levels of endogenous cortisol.	Activated by stress levels of endogenous cortisol.
90% occupied under 'normal' baseline conditions.	50% occupied under 'normal' baseline conditions.
Activation increases long-term potentiation, making neurons more responsive.	Increased activation overrides effect of mineralocorticoids by dampening down long-term potentiation. Also decreases utilisation of glucose throughout the brain.
Deficiency of mineralocorticoid-activation impairs selective attention and sensory integration, affecting downstream aspects of consolidation and retrieval.	Deficiency of glucocorticoid-activation only affects consolidation and retrieval aspects of memory.

1.2.6. Summary

The cortisol-response has the potential to promote learning and memory via activation of the two types of corticosteroid receptors. These are located throughout the brain, but in greater quantities in the hippocampus and frontal lobes. Consequently, this explains why the memory functions dependent on the integrity of these areas of the brain are sensitive to the effects produced (i.e., declarative and working memory). The MR- and GR-mediated effects on

memory are different. However, they do 'interact and proceed in a coordinated manner, linked in time to a particular stage in information processing' (De Kloet et al., 1999; p.424). Activation of the MRs is important in memory formation through sensory integration, whereas activation of the GRs is important during acquisition and consolidation. However, whereas activation of the GRs appears to be a pre-requisite for the long-term storage of information (De Quervain et al, 1998), enhanced occupation, such as that brought about during stress or following the administration of steroids, can be detrimental (Bremner, Randall & Scott, 1995; Mauri et al., 1993; Simmons, Do, Lipper & Laws, 2000). The evidence to support this is reviewed in the next section.

1.3 Review of the literature

Previous research has focussed on the effects of cortisol on memory in humans from two main perspectives. First, following changes in endogenous cortisol that have not been experimentally induced (e.g., as a result of psychological stress, age or pathology). Second, following changes in cortisol which have been experimentally induced (e.g., through the administration of synthetic exogenous corticosteroids, i.e., steroids). This section of the chapter presents a review of the literature produced from both perspectives. The effects of endogenous cortisol on memory are considered first.

1.3.1. Effects on memory following changes in endogenous cortisol

Table II (see page 47) presents a summary of the most notable studies conducted into the effects on memory following changes in endogenous

cortisol. These are considered in the following categories: stress-induced elevations; pathology-induced elevations; depression-associated elevations; and age-associated changes.

- *Stress-induced changes in cortisol*

Elevations in cortisol levels can occur either during stress (Seeman et al., 1997, or in anticipation of stress (Lupien et al., 1997), and studies have been carried out looking at the effects of experimentally-induced stress on memory using a variety of stressors. In 1996, Kirschbaum et al. investigated the effects of stress-induced changes in cortisol on memory by subjecting healthy adults to the Trier Social Stress Test (TSST). The TSST is a five minute public-speaking task followed by a five minute arithmetic task; both tasks are performed in front of an audience. Previous studies have shown that the administration of the TSST can reliably induce psychological and endocrine stress responses in many different healthy-adult populations (Kirschbaum, Pirke & Hellhammer, 1993; 1995). Indeed, it has been established that peak cortisol levels are normally obtained using the TSST thirty minutes after stress onset (Kirschbaum et al., 1993). By using an immediate test of declarative memory ten minutes following exposure to the TSST, Kirschbaum et al. found that those participants who produced the higher cortisol response showed the poorest verbal recall. They also found that mean cortisol levels were significantly lower five minutes before the administration of the stressor than after it. This suggests that the rise in cortisol occurred in response to the stressor as opposed to in anticipation of it.

Lupien et al. (1997) also used the TSST to compare the effects of a stressful (i.e., the TSST) versus a non-stressful (i.e., an attentional task) condition on the memory performance of healthy elderly adults. In addition to the two conditions, they also investigated the effects of cortisol on two different types of long-term memory: declarative memory (i.e., the conscious recollection of learned information); and procedural memory (i.e., the retrieval of information without conscious or explicit access). The results showed that the stressful condition significantly decreased declarative memory performance, whereas the non-stressful condition did not; procedural memory was not affected by either condition. In addition, although there was a slight increase in declarative memory performance at the second phase of testing (a potential effect of practice) this increase was not significant. The results of this study, therefore, suggest that the detrimental effects of stress on declarative memory can be protracted.

Individual differences in response to stress (i.e., high and low responders reported by Kirschbaum et al.) have also been identified by others (Bohnen, Houx, Nicholson & Jolles, 1990; Lupien et al., 1999). Lupien et al. identified high-responders as those participants who produced higher cortisol levels sixty minutes prior to the stressor; the low responders did not show any increase in cortisol levels until twenty-five minutes beforehand. They found that the stressed high responders performed significantly worse than the stressed low responders in declarative memory performance; no difference was found between each group in the non-stressful condition. Consequently, in contrast to Kirschbaum et al., they implied that it is the cortisol response

produced in anticipation of the stressor, rather than the actual stressor per se., that can have the detrimental effect on memory.

Bohnen et al. (1990) investigated the effects of stress-induced cortisol levels on cognition by exposing a group of healthy adults to four hours of continuous mental activity. They too classified participants as either high or low responders, based on the magnitude of their cortisol responses to tasks measuring aspects of verbal memory, concept shifting and divided attention. The results of this study showed that the high responders performed significantly worse than the low responders on the verbal memory task; indeed, the no/low cortisol responders did not present any effects of stress on memory at all. Like Lupien et al., Bohnen et al. also claimed that the detrimental effects on memory were in response to the anticipation of stress.

Another acute stressor paradigm used in this field is examination stress. However, the effects of this have not been so reliable. Indeed, according to Malarkey, Pearl, Demers et al., (1995), the 'unique nature' of the stressor can have a significant influence on the effects on memory produced. Vedhara, Hyde, Gilchrist et al. (2000) looked at the effects of examination stress on the memory performance of a group of undergraduates. They found that, although the exam period was associated with an increase in perceived stress levels, it was also associated with a reduction in salivary cortisol levels and enhanced free recall memory performance.

One explanation for these anomalous findings may be in the observation that repeated exposure to a stressor, followed by sufficient time to recover before being exposed to further stressors, can result in rapid habituation in certain individuals (Epel et al., 1998). In humans, cortisol

usually habituates after the first exposure to repeated stressors (Gunnar, Connors & Isensee, 1989 ;Levine, 1978; Mason, Brady & Tolliver, 1968). However, certain individuals, such as hypertensives and men with negative psychological traits (e.g., high aggression or hostility levels) can take longer to adapt (Al Absi & Lovallo, 1993; Kirschbaum, Pirke et al., 1995). Reduced habituation to recurrent events has also been identified in participants scoring high on perceived stress, anxiety and depression (Van Eck, Berkhof, Nicholson & Sulon, 1996). An inability to adapt to mild, recurrent stress can also cause intermittent stress to develop into chronic stress arousal (Epel et al., 1998); this is when the effects of cortisol can be most harmful to health. Epel et al. (1998) measured the cortisol levels of women exposed to three consecutive laboratory stress sessions. These comprised tasks which required solving difficult math and visuospatial problems, and delivering a speech. Each session lasted for three hours and started at the same time in the afternoon, thus controlling for any additional effects of time of day (e.g., levels of arousal or fatigue). As predicted, Epel et al. found that the women's cortisol habituation to stress occurred after first exposure to the repeated stressors. However, this was only observed in two of the four psychological subscales assessed (i.e., in the sub-scales designed to measure Appreciation of Life and Spiritual Growth; it was not observed in the sub-scales designed to measure New Possibilities and Relating to Others). Consequently, the authors suggested that the results found may have been due to chance. Indeed, the authors also went on to suggest that this study should be replicated using a more representative sample of the general population and with more naturalistic stressors.

It has also been shown that facile adaptation to stress can lead to increased learning and psychological resilience. For example, rats exposed to increased handling or mild shock when young, became much more resilient in later life (Levine & Brush, 1967). Although the same has not been reported in humans, these studies have been used as an argument for exposing children to brief manageable stressors rather than oversheltering them (Epel et al., 1998). This 'adaptation' to recurrent stressors may also explain why other researchers did not identify any significant increase in cortisol levels in students from baseline to examinations (Glaser, Pearly, Kiecolt-Glaser & Malarkey, 1994; Vedhara, Hyde, Gilchrist et al., 2000).

- *Pathology-induced changes in cortisol*

Cortisol levels also increase endogenously in patients suffering from hypercortisolaemia (i.e., persistently high levels of cortisol produced as a result of a increased activity of the HPA axis). Hypercortisolaemia is also experienced by patients with Cushing's Syndrome (Starkman, Gebarski, Berent & Schteingart, 1992) and often in patients with depression (Dinan, 1994).

The effects of pathology-induced increases in cortisol on memory performance were examined by Mauri et al. (1993). They compared the memory performance of a group of Cushing's patients with those of neurological patients with peripheral, but not central nervous system (CNS) disorders. In comparison to the controls, they found that the Cushing's patients were significantly impaired in verbal short- and long-term memory. They did not, however, show any significant impairments in either selective

attention or semantic memory. Similar findings to this have also been reported by other researchers (Martignoni et al., 1992).

There is also some evidence for an association between hypercortisolemia, impaired memory performance and reduced hippocampal volume. This illustrates how the effects of cortisol can be physiological as well as psychological. For example, patients with Cushing's Syndrome suffering hypercortisolaemia have shown reduced hippocampal volume and memory impairments (Starkman et al., 1992). It has also been shown that these detrimental effects can be reversed with treatment (Starkman et al., 1999). Similarly, patients with Post Traumatic Stress Disorder (PTSD) secondary to wartime experiences have also shown decreases in hippocampal volume (Bremner et al., 1995; Gurvits et al., 1989; Gurvits et al., 1996) and associated deficits in hippocampal-dependent memory (i.e., verbal memory - Bremner, Scott & Delaney, 1993; Bremner, 1999; Moradi, Doost, Taghavi et al., 1999). Bremner et al. (1995) found that PTSD patients had significantly smaller right hippocampi (8% smaller) compared with controls, but showed no difference in the volume of the other brain regions (i.e., the caudate and temporal lobes). Their short-term verbal memory deficits were also similar to those previously associated with smaller right hippocampal volume. Bremner et al. (1997) and Stein, Koverola & Hanna (1997) found similar results in a group of PTSD patients whose condition occurred as a result of childhood physical and sexual abuse.

There are, however, three main problems with the interpretation of these results. First, PTSD has been associated with lower 24-hour cortisol levels compared to controls (Bremner et al., 1993), although it should be noted

that this result has not been replicated universally. Hippocampal atrophy is also normally associated with increased cortisol levels, which suggests that the volume loss presented by these PTSD patients may have arisen either prior to, or as a result of, the trauma itself. Second, only 10-20% of patients exposed to combat trauma actually develop PTSD. This suggests that patients who succumb to PTSD may, pre-morbidly, have smaller hippocampi. Third, no-one has actually carried out post-mortems on patients with PTSD to confirm an associated loss of neurons. There is, however, evidence to show that patients with PTSD show an exaggerated negative feedback response to dexamethasone (Yehuda, Southwick & Krystal, 1993; Stein, Koverola & Hanna, 1997), and increased numbers of GRs in comparison to healthy controls (Yehuda, Kahana & Binder-Byrnes, 1995). Thus, the detrimental effects of cortisol identified by Bremner et al. may have either preceded, or been a consequence of, the trauma.

Although there is increasing evidence to suggest a connection between cortisol, hippocampal atrophy and cognitive performance, the 'underlying mechanisms' are not clearly understood. Consequently, there is great need to establish animal models which mimic these neuropathological processes (Ohl, Michaelis, Vollmann-Honsdorf et al., 2000). Indeed, Ohl et al. recently carried out a study looking at the connections between corticosteroids, hippocampal atrophy and hippocampus-mediated memory performance in two groups of male tree shrews. For four weeks, one group received cortisol treatment whilst the other group were subjected to psychological stress. The results showed detrimental effects on memory with an associated trend for reduced hippocampal volume in both groups of shrews. Furthermore, after a

seven week recovery period, traces of these effects still remained.

- *Depression-associated changes in cortisol*

Increased HPA axis activity, associated with increased levels of cortisol, can also occur in some patients with depression (Stokes, 1995). However, as with PTSD, it remains unclear whether this phenomenon precedes, or is a consequence of, the depression. Dinan (1994, 1996) suggested that the HPA 'overdrive' brought about by chronic stress in 'susceptible' people may be the 'core aetiological feature of depression'. Patients with major depression have also shown elevations in cortisol associated with impaired cognitive performance. For example, Rubinow, Post, Savard & Gold (1984) identified a significant correlation between cortisol levels and the number of errors in the Halstead Category Test. Cognitive impairment in depressed patients has also been correlated with 24-hour urinary-free cortisol levels (Rubinow et al., 1981).

Sheline, Sanghavi, Mintun & Gado (1999) used magnetic resonance imaging (MRI) to compare the direct effects of depression on hippocampal volume in medically healthy women with a history of recurrent major depression (i.e., ex-depressed female patients tested years and decades after their depression had gone). Although depression may not have been the cause of the atrophy, Sheline et al. found that the women with a history of depression had smaller hippocampal volumes bilaterally compared with controls. They also scored lower on the verbal memory tasks compared to their healthy controls. This suggests, therefore, that their hippocampal atrophy was related to this aspect of cognitive functioning. In addition, as this atrophy was still

present years after the last depressive incident, this suggests that depression-associated atrophy may not be reversible. This is in contrast to that seen in Cushing's patients, whose hippocampal atrophy reversed with treatment (Starkman et al., 1999). Sheline et al. also found no significant relationship between hippocampal volume and age in either the ex-depressed or control group. As the age of the participants ranged from 23 – 86 years, this does not support the age-related decreases in hippocampal volume previously identified by others (Lupien et al., 1994, 1998; Lupien & Forget, 1998). Sheline et al. did, however, identify a significant relationship between hippocampal volume and total lifetime duration of depression. It is important to note, however, that not all depressives secrete cortisol abnormally (Stokes, 1995). In addition, this study did not reveal whether the women showing atrophy years later were the ones who had had high cortisol levels when depressed.

- *Age-associated changes in cortisol levels*

Increased HPA activity has also been reported in the elderly (Heuser, Gotthardt, Schweiger et al., 1994;O'Brien, Schweitzer & Ames, 1994), with hippocampal atrophy loss of up to 40% associated with both normal ageing and Alzheimer's Disease (De Leon et al., 1996; West, Coleman & Flood, 1994). This has also been shown to be more prominent in females (i.e., there is evidence for gender differences in HPA activity). The ageing brain is also particularly susceptible to the detrimental effects of cortisol. This has been explained by a decrease in plasticity of the hippocampal receptors (Keenan, Jacobson & Soleymani, 1995) which, in turn, reduces the ability to down-regulate any increase in cortisol levels. Consequently, the elderly are at

greater risk for increased cognitive impairment and show a higher cortisol response to stress (Meaney, Gaudreau & Sharma, 1995). Indeed, Harman (1989) suggests that if rising cortisol levels were blocked or decreased in the elderly, this could have a very important impact on the ageing process.

The effects of cortisol on memory are analogous to those produced by normal ageing (Seeman et al., 1997). For example, it was found that the degree of declarative verbal memory deficits identified in Cushing's patients was similar to those in healthy matched controls aged 15 years older (Forget, Cohen, Somma & Lacroix, 1996). Increased cognitive impairment was also reported in older Cushing's patients compared to younger ones aged less than 45 years (i.e., the effects of age are additive). An age-related increase in cortisol has also been associated with declines in both verbal (Sharma, Turken & Schwartz, 1995) and nonverbal (Lupien et al., 1995) memory, with performance in tasks of recall and recognition impaired the most (Craik, 1992).

There is also some evidence to show that reductions in hippocampal volume and memory decline in the elderly are linked (Golomb et al., 1993). In rats, this has been explained by an age-related loss of cells that are necessary for normal HPA negative feedback (O'Donnell, Larocque & Seckl, 1994). In humans, a group of elderly adults with significant acute cortisol elevations was found to have reduced hippocampal volumes (by 14%) and significantly impaired hippocampus-dependent memory in comparison to elderly adults with decreasing and/or currently moderate cortisol levels (Lupien, de Leon, de Santi et al., 1998; Lupien et al., 1994). Similar results have been shown by the longitudinal MacArthur field study of successful

ageing, which identified a positive relationship between 12-hour urinary free cortisol excretion and declines in memory performance (Seeman et al., 1997). Lupien et al. (1994) also found that elderly adults with high baseline cortisol levels and whose cortisol levels had increased significantly over four years were impaired on tasks of declarative memory and selective attention. This was in comparison to elderly adults showing either a decrease in cortisol levels over years, or an increase in cortisol levels with moderate current basal cortisol levels.

Although these data imply that the detrimental effects of cortisol on hippocampal neurones may be one explanation for the age-related decline in cognitive function (McEwen, 1998), however, the relationship between cortisol levels and age is heterogenous. Indeed, following the examination of baseline cortisol levels in three sub-groups of elderly volunteers over a 3-6 year period, Lupien et al. (1997) found that, although cortisol levels increased with age in one sub-group, they decreased in a second group and remained stable in a third; individual differences in weight, height, body mass index, pulse, blood pressure and glucose had been controlled for in this study. Consequently, just as there are differences in how individuals respond to stress, there may also be a significant variation in HPA functioning in the elderly (Lupien et al., 1996).

Table II : Summary of the reviewed studies investigating the effects of endogenous cortisol on memory

STUDY BY:	TARGET POPULATION:	FINDINGS:
EVIDENCE SUPPORTING : How stress-induced changes in cortisol affect declarative memory performance		
Bohnen et al., 1990	Healthy adults	High cortisol-responders performed significantly worse on a task of verbal memory following four hours exposure to continuous mental activity. Low cortisol-responders showed no effects.
Kirschbaum et al., 1996	Healthy adults	Found that high cortisol-responders showed the poorest verbal recall in comparison to low cortisol responders. Claimed rise in cortisol was in response to the stressor as opposed to the anticipation of it.
Lupien et al., 1997	Healthy elderly adults	Compared effects of stressful with non-stressful condition. Identified decrease in declarative memory performance following stressful condition only. Claimed rise in cortisol was in response to the anticipation of stressor as opposed to the stressor itself. In addition, high cortisol-responders performed significantly worse than low cortisol-responders.
Vedhara et al., 2000	Healthy adults (students)	Looked at effects of examination stress on memory performance. Although there was an increase in perceived stress levels, this was associated with a reduction in cortisol levels which, in turn, was associated with enhanced memory performance.
EVIDENCE SUPPORTING : How pathology-induced changes in cortisol affect declarative memory performance		
Bremner et al., 1995; 1997 Results supported by: • Gurvits et al., 1996 • Moradi et al., 1999 • Stein et al., 1997	Patients with Post Traumatic Stress Disorder	Found 8% -12% smaller right hippocampal volumes in Post Traumatic Stress Disorder patients compared to controls. No differences in the volumes of other brain regions were found. Also found associated short-term memory deficits with reduced hippocampal volumes.
Bremner & Narayan, 1998	Trauma patients	Found that the stage at development at which trauma takes place may influence nature of memory deficits and hippocampal atrophy.
Mauri et al., 1993 Results supported by : • Martignioni et al., 1992	Patients with Cushing's Syndrome.	Found Cushing's Syndrome patients, suffering from hypercortisolemia, had impaired verbal short- and long-term memory in comparison to non-Central Nervous System neurological controls. No significant impairments were found in either selective attention or semantic memory.
Ohl et al., 2000	Male tree shrews	Found decreased memory performance with an associated trend for reduced hippocampal volume in male trees shrews following four weeks treatment with cortisol/psychological stress. Traces of effect were still present seven weeks after recovery period.
Simmons et al., 2000	Patients with Cushing's Syndrome	Identified relationship between cortisol levels, memory deficits and reduced hippocampal volume.
Starkman et al., 1992	Patients with Cushing's Syndrome	Reported decreased hippocampal volume and associated memory impairment as a result of high cortisol levels.
Starkman et al., 1999	Patients with Cushing's Syndrome	Decreased hippocampal volume and memory impairment reversed with treatment.
EVIDENCE SUPPORTING : How changes in cortisol brought about by depression can impair declarative memory performance		
Dinan, 1996	Patients with depression	Found nearly all depressed patients showed impaired memory performance. Suggests that HPA overdrive brought about by chronic stress in susceptible people may be the core aetiological feature of depression
Rubinow et al., 1981	Patients with depression	Identified a significant correlation between level of cognitive impairment and 24-hour urinary-free cortisol levels.
Rubinow et al., 1984	Patients with depression	Identified significant correlation between cortisol levels and no of errors in Halstead Category test.

Sheline et al., 1999	Medically healthy, post-depressed women	Found post-depressed women had small hippocampal volumes compared with controls and also produced lower verbal memory scores. Also found a significant relationship between hippocampal volume and total lifetime duration of depression.
EVIDENCE SUPPORTING : How age-associated changes in cortisol levels can impair declarative memory performance		
Craik & Jennings, 1992	Healthy elderly adults	Age related increase in cortisol associated with deficits in tasks of recall and recognition.
Forget et al., 1996	Patients with Cushing's Syndrome	Found that levels of explicit verbal memory deficits in Cushing's patients were similar to healthy matched controls aged 15 years older.
Golomb et al., 1993	Healthy elderly adults	Identified relationship between reduction in hippocampal volume and memory decline.
Lupien et al., 1994; 1998	Healthy elderly adults	Identified a significant relationship between prolonged cortisol levels, reduction in hippocampal volume and deficits in explicit memory. Also found that elderly individuals with significant increases in cortisol levels over 4 years and high current basal cortisol levels were impaired on tasks of declarative memory and selective attention.
Lupien et al., 1995	Healthy elderly adults	Found that age-related increases in cortisol levels were associated with deficits in both explicit memory and selective attention.
Lupien et al., 1996	Healthy elderly adults	Identified considerable variation in HPA functioning in the elderly, i.e., the relationship between cortisol levels and age is heterogenous.
EVIDENCE SUPPORTING : How age-associated changes in cortisol levels can impair declarative memory performance (continued)		
Seeman et al., 1997	Healthy elderly adults	Identified a significant relationship in 24-hour free cortisol excretion in urine and performance on tests of delayed verbal recall in females. Also, found that females who exhibited increases in cortisol secretion over a 2.5 year follow-up were more likely to show declines in memory performance. No significant associations were found among the men. Effects of memory were analogous to those produced by normal ageing.
Sharma et al., 1995	Healthy elderly adults	Found that age-related increases in cortisol levels were associated with declines in verbal memory.

1.3.2. Summary

Research investigating the effects of endogenous cortisol on memory has identified several factors that can influence the production of cortisol and its associated effects on memory. Studies examining stress-induced changes in cortisol have identified a clear negative relationship between cortisol levels and hippocampal-dependent memory. It is not clear, however, whether it is the response brought about in anticipation of stress or the response brought about by the stressor per se. which can have the most harmful effects. The nature of the stressor and habituation to stress can also produce different effects. For example, an examination period was not found to be a reliable

stressor, as measured by cortisol levels, in students even though they perceived themselves as feeling stressed.

Studies investigating the effects of pathologically-induced elevations in cortisol have identified both indirect and direct associations with increased cortisol levels (i.e., hippocampal-dependent memory deficits and reduced hippocampal volume, e.g., Bremner et al., 1995; Mauri et al., 1993; Simmons et al., 2000). With pathological conditions, however, it can be difficult to discriminate between the cognitive deficits due to the underlying pathology versus those due, primarily, to changes in cortisol levels (Wolkowitz et al., 1990).

Several studies looking at the effects of ageing and dementia have also identified a relationship between the degree of memory impairment and hippocampal volume. However, whilst these data may increase our understanding of the ageing process, as with differences in how people respond to stress, the relationship between cortisol and age in individuals is heterogenous. As discussed briefly, there are also gender differences which may affect the interpretation of these results.

The effects of cortisol on memory have also been investigated following the administration of synthetic exogenous corticosteroids (i.e., steroids). The effects of exogenous cortisol on memory are considered next.

1.3.3. Effects on memory following changes in exogenous cortisol

Table III presents a summary of the most notable studies to have been carried out looking at the effects on memory following the administration of steroids.

These are considered in the following categories: steroid therapy; the administration of steroids to healthy populations; and the effects of steroids on working memory. Studies investigating the effects of steroids on memory have also identified several important factors which can modify the effects on memory produced. These are also presented in Table III and are reviewed in this section in the following categories: the use of different testing protocols; selective effects of steroids; effects of dosage; age by duration effects; and effects of steroids on circadian variation.

- *Steroid therapy*

Therapeutic doses of synthetic steroids, like hydrocortisone, prednisone and dexamethasone, are used to treat many medical conditions. For example, replacement doses are used for the treatment of adrenal insufficiency (e.g., Addison's Disease) and pharmacological doses are used for various inflammatory states, such as bronchial asthma and rheumatoid arthritis (Schimmer & Parker, 1996). Investigations into the effects of steroids on memory performance have been carried out, with similar findings to those observed following changes in endogenous cortisol levels.

Keenan et al.(1995) investigated the effects of chronic prednisone treatment on memory performance in patients with rheumatic disease (without CNS involvement). These patients had been treated with 15 mg prednisone daily for at least one year. Compared to matched controls (i.e., patients treated with alternative therapies, such as gold) the prednisone group performed significantly worse on tests of paragraph recall and list learning. In addition, although the level of effect was not influenced by the dose of steroid

administered, the effects were more pronounced after short-term than long-term use. This implies that the detrimental effects of steroids may reach an optimum point and/or that long-term use can result in habituation to the effects.

In a similar study one year later, Keenan et al. (1996) assessed the declarative and procedural memory performance of patients with systemic disease (without CNS involvement) and their matched controls. The patients had been treated with between 5 to 40 mg prednisone daily for at least one year. The results of this study showed no difference between the two groups on procedural memory. However, the prednisone-group performed significantly worse than the controls on declarative memory (i.e., only hippocampal-related memory performance was affected). The older patients in the study also showed greater memory deficits with less protracted treatment than the younger patients. This implies that the threshold for the harmful effects of steroids may decrease with age.

Keenan et al. (1996) also carried out a three-month prospective study on a group of patients treated with 40-60 mg of prednisone daily for systemic disease (again without CNS involvement). Compared to matched controls, these patients produced significantly lower delayed-paragraph recall scores. In addition, the detrimental effects on memory became apparent one week after treatment began (i.e., the effects of steroids were time-related as well as dose-related).

Children with asthma who do not respond to bronchodilators are sometimes treated with prednisone and, as a consequence, may be vulnerable to any effects of steroids. Such effects include suppression of the HPA axis

(Chang & Tam, 1991) and growth suppression, which have been identified in children following even moderate doses of treatment (Kannisto, Korppi, Remes & Voutilainen, 2000). Steroid-related deficits in visual and verbal memory in asthmatics have also been reported (Sausa, Stump & Chai, 1986) again related to dose. For example, Bender Lerner & Koilasch (1998) and Bender, Lerner & Poland (1991) identified verbal memory impairments in children treated with high-, but not low-, dose therapy (i.e., 61.4 vs. 6.97 mg/day respectively).

There is, however, conflicting evidence that shows no significant differences in memory performance between children with asthma (and treated with steroids) and healthy matched controls (Rietveld & Colland, 1999; Weldon & McGeady, 1995). For example, Rietveld and Colland compared school performance, including memory concentration, between children (aged 10 – 13 years) with severe asthma and healthy matched controls. They found that the performance of the asthmatics did not deviate significantly from that of the controls. In addition, it has been suggested that any differences in memory performance between children with asthma treated with steroids, and healthy controls, may be due to other non-neuropsychological factors (e.g., the child's socioeconomic status and any adverse effects of asthma on learning; Annett & Bender, 1994).

It has been shown that the time that psychological stress occurs in relation to life cycle can also have implications for childhood development and ageing (Bremner & Narayan, 1998). Consequently, if similar effects can occur following the administration of steroids, the clinical implications for using corticosteroid-therapy on children are much greater.

- *The administration of steroids to healthy populations*

As with pathology-induced increases in cortisol, one of the major problems with data obtained from any clinical-population treated with steroids is the difficulty in discriminating between the effects produced by steroids and those due to the pathology alone. Indeed, some of the memory deficits which have previously been attributed to the effects of steroids may, in fact, be those due to other aspects of the pathology (Deptula, 1983; Wolkowitz et al., 1990). For example, both Deptula and Wolkowitz et al. showed that the cognitive impairments identified in depressed patients (i.e., errors of commission in verbal memory) were similar to those found following the administration of steroids to normal healthy controls. However the effects of depression alone may incur cognitive impairments. Consequently, to control for this, the effects of steroids on memory performance have also been carried out following the administration of steroids to healthy participants and other non-CNS involved clinical populations. In general, these have also produced similar results to those identified in clinical populations (Beckwith, Petros, Seagrove & Nelson, 1986; De Quervain et al., 2000; Fehm-Wolfsdorf, Reutter, Zenz & Born, 1993; Fehm-Wolfsdorf, Scheible, Zenz, Born & Fehm, 1989; Kirschbaum et al., 1996; Lupien et al., 1995, 1999; Newcomer, Selke, Kelly, Parras & Craft, 1995; Schmidt, Fox, Goldberg, Smith & Schulkin, 1999; Wolkowitz et al., 1990, 1993).

The first study to explore the effects of acute increased changes in cortisol levels using steroids on memory in a healthy population was conducted by Beckwith et al. (1986). In this study, different doses of hydrocortisone (i.e., 5, 10, 20 and 40 mg) were administered to healthy young

males to investigate the effects on both short- and long-term memory. Sixty minutes following administration, Beckwith et al. found that, whereas each of the doses facilitated the recall of words during the first presentation of word lists, the effects were dose-dependent; only the highest dose continued to enhance recall when additional lists of words were presented. A positive relationship between performance and the amount of practice given on each task was also found. However, Beckwith et al. found no direct association between hydrocortisone levels and memory performance. Indeed, they attributed this to changes in motivation and arousal, rather than to any specific enhancement of memory function. There have, however, been other methodological explanations for this lack of effect. For example, Beckwith et al. used glucose as their control condition. They also added glucose to each of the doses of hydrocortisone they administered. Glucose can enhance cognitive performance (Benton, Owens & Parker, 1994; Parker & Benton, 1995; Korol et al., 1995; Parsons & Gold, 1992) and, according to Lupien & McEwen (1997) this may explain the positive effects they obtained.

In support of this explanation, Kirschbaum et al. (1996) used a similar protocol to Beckwith et al., but without the addition of glucose; they used saline as a control. They also administered 10 mg of hydrocortisone only. In contrast to the placebo group, the results of this study showed a significant decrease in cued-verbal recall and spatial thinking in the hydrocortisone group. They also found no effects on the non-hippocampal-priming task. A comparison of the Beckwith and Kirschbaum studies highlights the importance of considering any differences in testing protocols when interpreting and comparing results.

Schmidt et al. (1999) examined the effects of acute treatment with steroids in a group of healthy males who self-administered high doses of prednisone (160 mg) every morning for a total of four days. By using a simple recall task to test the effects on declarative memory, Schmidt et al. found that the treatment group recalled fewer objects on the fourth day following treatment (i.e., day 8) compared with matched controls. The treatment group also performed more poorly on a delayed recall task one hour after treatment, suggesting short-term, as well as longer-term, effects on memory.

Newcomer et al. (1999) carried out a recent study using steroids to replicate the effects of several days exposure to different levels of both physical and psychological stress. To produce these stress-equivalent cortisol levels, healthy volunteers received one of two fixed oral doses of hydrocortisone (i.e., either 40 mg/day or 160 mg/day). The 40 mg dose corresponded to the levels of cortisol which might be secreted during a minor medical procedure (e.g., getting stitches or having a skin growth removed), whereas the 160 mg dose corresponded to the levels of cortisol which might be secreted after events like abdominal surgery. The doses of hydrocortisone were administered in split doses (i.e., by capsules, twice daily) for four days. These split doses were administered to approximate circadian variation in cortisol secretion (i.e., high in the morning and low in the evening). Memory testing was carried out at 4 pm at baseline, at one and four days after treatment, and finally after a six-day washout period.

The results showed a significant interaction between time and treatment condition for paragraph recall, but only after administration of the highest dose of hydrocortisone. Thus, it appears that maximal levels of stress.

as replicated by 160 mg/day hydrocortisone, can impair declarative memory. No such deficits were found under the moderate stress condition (i.e., 40 mg/day). In addition, after receiving the six-day 'treatment washout', memory performance returned to untreated levels. Newcomer et al. interpreted their results as suggesting 'that it would take several days of stresses like major surgery or severe psychological trauma in order for cortisol to produce memory impairment' (p.352). They also claimed that, as the effects were reversible, they did not believe the effects on memory demonstrated by their study 'were the part of any process associated with loss of neurons or permanent damage in the hippocampus or other brain structures' (p.352). They did, however, suggest that if these high levels were sustained, the hippocampal neurons may become vulnerable to other types of injury (e.g., atrophy) and that similar effects may occur after long-term exposure to slightly lower levels. To date, studies looking at the effect of long-term exposure with slightly lower levels of cortisol have not been carried out.

The effects of steroids on memory can also depend on the timing of the treatment relative to learning and testing. A recent study by De Quervain et al. showed that the acute administration of 25 mg hydrocortisone given 24 hours after learning but one hour before delayed free-recall testing, significantly impaired recall performance compared to controls. It did not, however, affect recognition memory. In contrast, however, the administration of 25 mg hydrocortisone given either pre-learning or immediately post-learning had no effects on either recall or recognition performance (De Quervain et al., 2000). The participants' subjective ratings of stress levels one hour following treatment were also not affected. De Quervain et al. interpreted these results

as showing that cortisol impairs memory retrieval specifically and that, consequently, this could have implications for the reliability of information remembered under times of stress (e.g., during examinations, courtroom testimony or performance in combat).

- *The effects of steroids on working memory*

As mentioned previously, a recent study by Lupien et al. (1999) using steroids suggested that working memory may be more sensitive to the acute changes of cortisol than declarative memory. Indeed, this suggestion has been offered as an explanation for the detrimental effects of steroids reported during the acquisition and consolidation of information.

Lupien et al. administered differing doses of hydrocortisone (i.e., approximately 1.2 mg, 8.6 mg and 16.6 mg) to young healthy males. By using tasks of item-recognition, paired-association and continuous performance they identified significant acute effects of the highest dose of hydrocortisone on working memory, but with no significant effects on declarative memory or arousal/vigilance. The effects of chronic elevations of steroids on working memory were not reported. It is important to note, however, that this is the only study in this category and, at the time of writing this thesis, the results had not been replicated.

1.3.4 Summary

Research investigating the effects of exogenous cortisol on memory has identified several factors concerning their effects on memory. First, many of the effects observed following the administration of steroids have been similar

to those produced following elevations in endogenous cortisol. Second, although there have been some discrepancies between results (e.g., in children treated for asthma), studies investigating the effects of steroid therapy have generally identified detrimental effects on declarative memory. Third, it appears that the elderly are more sensitive to the detrimental effects than younger adults. However, one of the problems with using clinical populations is discriminating the effects of steroids from those produced by other aspects of the pathology. Consequently, similar investigations have been carried out administering steroids to healthy populations and the results of these have been similar to those found in clinical populations. Finally, although at the time of writing this thesis the study by Lupien et al. (1999) had not been replicated, it appears that working memory may be more sensitive to the effects of acute changes in cortisol than declarative memory. It is, therefore, important that tasks of recall, as well as recognition, are used to identify at which stage during the declarative memory process the effects of steroids occur.

1.3.5. Factors shown to modify the effects of steroids on memory

A number of factors have been found to modify the effects of steroids on memory. These include: the use of different testing protocols; the use of different cognitive measures; the different effects of different steroid-types; time-course differences in effects of steroids; the selectivity of steroid-related impairments; age by duration effects of corticosteroids; and the effects of steroids on circadian variation.

- *The use of different testing protocols*

As mentioned previously, one of the explanations for the discrepant results identified following the administration of 10 mg hydrocortisone by Beckwith et al. (1996) compared with those found by Kirschbaum et al. (1996) was the use of different placebos. A second explanation, however, relates to the differences in time incorporated between learning and testing. Beckwith et al. tested recall performance immediately after learning. Alternatively, Kirschbaum et al. incorporated a delay between learning and testing. A period of delay between learning and recall gives greater assurance that long-term memory is being assessed (Wolkowitz, Reus, Canick, et al., 1997); this may explain why Kirschbaum et al. reported detrimental effects on memory whereas Beckwith et al. did not. Comparison of these two studies, therefore, highlights the significant effects that even the most subtle of differences in protocols can produce.

A discrepancy between results, even though a similar protocol was used, was also reported by Lupien et al. (1999). As part of their study investigating the effects of acute changes in cortisol levels on memory using steroids, Lupien et al. used a similar declarative memory task to Kirschbaum et al. (1996), but they instructed the participants to complete the task using intentional encoding. Intentional encoding is when participants are made aware during the instructive phase that they will later have to recall the words from the word list they are given to learn. Kirschbaum et al. used incidental encoding (i.e., their participants were not made aware of this). Incidental encoding has been shown to lead to lower recall when compared to intentional encoding because of the poor elaboration performed on the material at the time

of encoding (Mandler, 1980). The length of declarative memory task used by Lupien et al. was also different to the one used by Kirschbaum et al.; this was because the two studies were interested in the effects of steroids on different stages of the long-term memory process. More specifically, Lupien et al. was interested in the effects of acute changes in cortisol on working memory and declarative memory. Consequently, the declarative memory task they used was short as they did not want it to overload the limited processing capacity of working memory. In contrast, Kirschbaum et al. was interested in the effects of acute changes in cortisol on the later stages of declarative memory. Consequently, their task was a much longer task.

- *The use of different cognitive measures*

Another explanation for the difference in effects of cortisol on memory has been the use of different cognitive measures (e.g., De Quervain et al., 2000). The use of different tasks to assess the same aspects of memory can make comparisons across studies difficult.

The effects of cortisol on declarative memory have been identified using a variety and range of different cognitive measures. These include tasks of: recognition and paragraph recall (Lupien et al., 1994); cued recall, in particular recall of new associations and not pre-existing ones to test acquisition and recall (Lupien et al., 1994, 1995); verbal memory (Starkman et al., 1992); delayed recall (Lupien et al., 1998); and free recall (Fehm-Wolfsdorf et al., 1993). However, studies which have used similar measures have not always found the same results. Indeed it has been argued that different memory tasks may 'address' different CNS mechanisms (Lezak,

1983). For example, as mentioned previously, Kirschbaum et al. (1996) and Lupien et al. (1999) both used similar tasks of declarative memory, but only Kirschbaum et al. observed any memory deficits. It has also been suggested that the tasks used to identify the differential effects of cortisol on memory may not have been sensitive enough to identify at which phase of the memory formation process these effects take place. This is particularly important if the treatments being administered affect more than one memory phase.

In two independent studies with healthy volunteers, Wolkowitz et al. (1990) compared the effects on memory produced following one single 1mg dose of dexamethasone with those produced following 5 days treatment with 80 mg/day prednisone. In the first study, free recall performance was tested one week prior to and one day following the administration of dexamethasone. Compared to controls, the treatment group produced significantly more intrusion errors. In the second study, a similar design was used but with a longer latency between learning and recall/recognition; as mentioned earlier, a delay between learning and recall more fully assures that this aspect of declarative memory is being tested. In this study, the treatment group was tested once during an initial 5-day placebo period, once after 4 days of prednisone and once again 7 days after the discontinuation of prednisone (i.e., on day 11). The treatment group performed significantly worse than their matched controls on word-detection and were found to misidentify distractor-as target-items. No significant differences were seen between both groups in free recall. In addition, Wolkowitz et al. found that by day 11 performance levels returned to normal (i.e., the detrimental effects were reversed).

Both of these studies identified a similar relationship between steroids and memory (i.e., an increase in the number of errors in a verbal declarative memory task). However, although the effects were the same, they were identified using different types of declarative memory tasks (i.e., a free recall task was used to investigate the effects of dexamethasone, whereas a recognition task was used to investigate the effects of prednisone). This highlights the importance of either using the same cognitive measures, or controlling for differences, when making comparisons across studies.

- *The different effects of different steroid-types*

Different types of steroids have preferences for different types of corticosteroid receptors. For example, fludrocortisone (a mineralocorticoid) has a preference for MRs, whereas dexamethasone (a glucocorticoid) has a preference for GRs. However, even different steroids with preferences for the same receptor-types have shown different effects on memory. For example, when Wolkowitz et al. (1990) administered prednisone (which has a preference for both receptor-types), they identified deficits during recall and recognition performance. Alternatively, when De Quervain et al. (2000) administered hydrocortisone (which also has a preference for both receptor-types), they reported deficits in retrieval performance, but with no effects in immediate recall or recognition. It is important to note, however, that there were a number of methodological differences between these two studies which might explain this difference in effect (e.g., the two studies used different time periods between the administration of steroids, learning and testing).

- *Time-course differences in effects of corticosteroids*

The time course for any cortisol-related effects on memory can also vary and whilst this may be related to the different affinities for the different corticosteroid receptors, it may also be related to how quickly the corticosteroids are penetrated by the brain and bind to the receptors (Coirini, Flores, Vega et al., 1994; Meijer et al., 1998). For example, cortisol enters the brain rapidly. In contrast, dexamethasone when given in low doses does not (Lupien & McEwen, 1997). This is because there is a pump that pumps dexamethasone out of the brain and thus, this reduces how effectively it is penetrated. These differences in binding rates can also determine whether the effects of cortisol are immediate or delayed. In some cases, the effects can be acute and rapidly depress the firing activity of neurons as early as minutes after initial exposure (Saphier & Feldman, 1988). For example, Kirschbaum et al. (1996) identified deficits in declarative memory sixty minutes following the administration of hydrocortisone. More commonly, however, the effects of steroids may not occur until hours/days after exposure (Pfaff, Silva & Weiss, 1971). For example, Newcomer et al. (1994) reported delayed effects in declarative memory following the administration of dexamethasone. Newcomer et al. administered different doses of dexamathasone (i.e., 0.5, 0.5, 0.5 and 1 mg) to healthy adults over a four day period (i.e., on days 0, 1, 4 and 11 respectively). By the fourth day only (i.e., day 11) deficits in verbal declarative memory (i.e., paragraph recall) were reported; there were no significant changes in levels of arousal or attention. Wolkowitz et al. (1990, 1993) also identified delayed effects of predisone on declarative memory. They administered high doses of prednisone (80 mg) to healthy adults aged

between 21 and 41 years and reported problems in their detection of target words by the fifth day only. These results may, however, be misleading as it appears that the first day the effects on memory were assessed was the fifth day following administration (i.e., they may have actually occurred earlier on).

Lupien et al. (1995) also identified delayed, but not immediate, effects of steroids during the consolidation phase of declarative memory. They administered acute infusions of either placebo (saline) or hydrocortisone (i.e., 40 μg/kg, 300 μg/kg and 600 μg/kg [equivalent to approximately 1.2 mg, 8.3 mg and 16.6 mg]) to four groups of healthy young controls. By using a cued recall task to test acquisition and recall, they identified deficits in recall performance by the fourth day only. Lupien et al. also identified a positive relationship between the level of deficit and dose of hydrocortisone infused.

Dose may also explain the time differences in the effects of steroids. For example, as a follow up to their earlier study in 1994, Newcomer et al. (1995) administered higher doses of dexamethasone to healthy adults (i.e., 1, 2, 3 and 4 mg/day). In this study they identified immediate as well as delayed effects during acquisition and recall performance. They also identified age differences in the effects produced. Although the young participants presented immediate as well as delayed effects, no effects at all were identified in the elderly group. This lack of effect may be related to the age-related, slow penetration of dexamethasone by the brain (Newcomer et al., 1994). If the elderly group had been treated with dexamethasone for a longer period than the younger ones, similar deficits may have been reported for both age groups (i.e., sufficient time should be allowed between administration, learning and testing to give the steroid time to enter the brain).

- *Age by duration effects of corticosteroids*

Keenan et al. (1995) identified a significant age by duration effect between younger and older participants who had received long-term treatment with steroids. The two groups comprised younger adults aged 45 years and below, and older adults aged 46-72 years. Both groups showed detrimental effects on declarative memory. However, in the older-age group only, these detrimental effects appeared to plateau after the first three years of treatment (i.e., there was no increase in detrimental effects after three years).

There are a couple of speculative explanations for these results. First, as older adults are more sensitive to the detrimental effects of cortisol, they may have become habituated to the chronic effects of steroids. Second, the additional effects of age may have been compounded by the age-associated effects on circadian variation (i.e., the timing of the circadian release of cortisol can change with age). It is important to note, however, that the results of this study had not been replicated at the time of writing this thesis and these explanations have not been supported.

- *The effects of steroids on circadian variation*

There is evidence to suggest that the effects of cortisol on memory may also be influenced by the time of day. As described earlier, cortisol is involved in the formation of circadian events, with levels that normally fluctuate throughout the day. During the 'awakening cortisol response' (30-45 minutes after waking), free cortisol levels increase two to three fold (Hucklebridge, Clow, Abeyguneratne, 1999). It has been suggested that this is to enable the body to prepare for the metabolic demands of the day. After waking, cortisol levels

then remain high in the morning (i.e., around 700 nMol/L at the peak), dropping to a minimum (i.e., less than 10 nMol/L) in the hour after midnight (Keenan & Kuhn, 1999). In 'normals' undergoing stress and in some patients suffering depression, these peak levels can increase to greater than 1000 nMol/L (Keenan & Kuhn, 1999). The administration of steroids, however, can suppress the normal circadian variation of circulating cortisol levels (Fehm-Wolfsdorf et al., 1993), which it does by inhibiting the negative feedback actions at the hippocampal, hypothalamic and pituitary levels. Indeed, the administration of steroids can completely block endogenous cortisol secretion (Huppertz & Pfuller, 1997) and, consequently, may mask any other time of day effects (e.g., arousal levels).

The effects on circadian variation were identified by Fehm-Wolfsdorf et al. (1993) when comparing the effects of endogenous versus exogenous corticosteroids on free recall and recognition performance at two times of day (i.e., at 09.00 hrs and at 18.00 hrs). In a 2 x 2 repeated measures design, each participant was also tested under each one of two further conditions: after receiving stress levels of hydrocortisone (50 mg), or placebo. All memory testing was carried out one hour following the administration of either hydrocortisone or placebo. The results revealed no significant differences in recall performance between the hydrocortisone and placebo sessions. In addition, although the participants' performance levels in the placebo condition were higher in the morning (when cortisol levels are normally high) compared to the afternoon, there was no significant difference between morning and afternoon performance levels in the hydrocortisone condition. Consequently, it appeared that the administration of hydrocortisone had

suppressed the normally improved memory performance in the morning, but had no effect when administered at night.

Apart from the Fehm-Wolfsdorf et al. study, however, previous studies looking at the effects of cortisol on memory have tended to control for the effects of diurnal variation. For example, Kirschbaum et al. (1996) always tested participants in the late afternoon; Lupien et al. (1996) tested at 13.30 hrs, and Newcomer et al. (1994; 1999) tested at 16.00 hrs. A study manipulating different levels of cortisol (both naturally and artificially) and testing different aspects of memory at different times of day would clearly provide important information regarding the inverted-U shaped relationship between types of steroids and cognitive performance.

Table III : Summary of studies reviewed investigating the effects of steroids on memory

STUDY BY:	TARGET POPULATION:	STEROID TYPE/ DOSE:	FINDINGS:
EVIDENCE SUPPORTING : The effects of steroid therapy on declarative memory performance			
Bender et al., 1988; 1991	Children with asthma	Prednisone - 61.4 and 6.97 mg/day	Identified deficits in verbal memory performance following high, but not low-dose therapy, i.e., effects were dose-related.
Keenan et al., 1995	Patients with Rheumatic Disease (without Central Nervous System involvement)	Prednisone – 15mg daily, for at least one year	Compared to matched controls, patients performed worse in tasks of paragraph recall and list learning, especially after short-term use; the effects were not influenced by dosage. Also identified a significant age by duration effect in elderly participants, but only up to the first three years.

Keenan et al., 1996	Patients with Systemic Disease (without Central Nervous System involvement)	Prednisone, between 5 & 40 mg daily, for at least one year	Compared performance on tasks of explicit and implicit memory between treatment group and matched controls. Found no difference in implicit memory performance, however treatment group performed significantly worse in explicit memory. Also found that elderly patients showed greater susceptibility to memory deficits with less protracted treatment.
Keenan et al., 1996	Patients with Systemic Disease (without Central Nervous System involvement)	Prednisone, between 40 – 60 mg daily for 3 months	Compared to matched controls, patients produced significantly lower delayed-paragraph recall scores. These effects were evident after one week of treatment.
Sausa et al., 1986	Children with asthma	Prednisone	Deficits in both visual and verbal memory have been reported consistently in this population.
Weldon & McGeady 1995 Results supported by: • Rietveld & Colland 1999	Children with asthma	Theophylline	Identified no significant difference in memory performance between children treated with steroids for asthma and healthy matched controls.
Wolkowitz et al., 1990	Patients with depression	Dexamethasone	Found that patients who did not suppress cortisol made significantly more false positives in a verbal memory task compared to depressed cortisol suppressors and normal controls.
EVIDENCE SUPPORTING : The effects on declarative memory produced following the administration of steroids to healthy populations			
Beckwith et al., 1986	Young, healthy males (students)	Hydrocortisone – 5, 10, 20 and 40mg	Found that all doses facilitated recall of first presented word lists, but only highest dose enhanced recall of additional lists. Also identified positive relationship between performance and amount of practice. Study confounded by use of glucose as placebo.

De Quervain et al., 2000	Young, healthy males	Hydrocortisone – 25 mg	Identified decreased retrieval during free recall after immediate and delayed testing; no effects were identified in recognition performance. Also found that administration of hydrocortisone immediately after presentation of the word list affected immediate recall, but had no effects on delayed recall, i.e., acquisition and not consolidation was affected.
Kirschbaum et al., 1996	Healthy adults	Hydrocortisone – 10mg	Similar to Beckwith et al. (1986) study, but using one dose of hydrocortisone and saline as placebo. Identified decreased performance in cued-verbal recall and spatial thinking tasks as a result of treatment.
Newcomer et al., 1999	Healthy adults	Hydrocortisone – either 40 mg/day or 160 mg/day	Identified a significant interaction between time and treatment condition for paragraph recall after administration of 160 mg hydrocortisone only. Effects reversed after 6-day washout treatment.
Schmidt et al., 1999	Healthy males	Prednisone – 160 mg every morning for 4 consecutive days	In comparison to control group, treatment group recalled fewer objects on 4th day following treatment (i.e., by Day 8). Also performed more poorly on delayed recall task one hour after treatment.
EVIDENCE SUPPORTING : The specificity of steroid-related declarative- memory impairments			
Wolkowitz et al., 1990	Healthy adults	Dexamethasone - one x 1mg dose	Identified increased number of intrusion errors in declarative memory tasks one day following treatment.
Wolkowitz et al., 1990	Healthy adults	Prednisone - 80mg/day for 5 days	Identified significant mis-identification of distractor-as- target items in recognition task following treatment. Performance returned to normal 7 days after discontinuation of treatment, i.e., detrimental effects were reversed.
EVIDENCE SUPPORTING : Delayed versus immediate effects of steroids on declarative memory			
Kirschbaum et al., 1996	Healthy adults	Hydrocortisone – 10mg	Identified almost immediate deficits in declarative memory performance 60 minutes following administration of treatment.

Lupien et al., 1996	Healthy adults	Hydrocortisone – 40, 300 or 600 μg/kg	Identified delayed, but not immediate, effects during the consolidation phase in acquisition and recall performance by fourth day only. Also identified positive relationship between level of deficit and dosage of hydrocortisone.
Newcomer et al., 1994	Healthy adults	Dexamethasone – 0.5/0.5/0.5 and 1 mg over 4 days	Identified decreased paragraph recall performance after fourth day only. No significant changes were identified in levels of arousal or attention.
Newcomer et al., 1995	Healthy adults	Dexamethasone – 1, 2, 3 and 4 mg over 4 days	Identified immediate as well as delayed effects during acquisition and recall performance. Also found age differences in effects, i.e., young presented immediate as well as delayed effects, whereas elderly presented no effects at all.
Wolkowitz et al., 1990; 1993	Healthy young adults	Prednisone - 80 mg/ day over 5 days	Identified a significant increase in the incorrect detection of target words by 5 days. However, memory performance had not been assessed prior to treatment.
EVIDENCE SUPPORTING : Effects steroids on working memory			
Lupien et al., 1999	Young, healthy males	Hydrocortisone – 40, 300 or 600 μg/kg	Identified deficits in working memory, but no effects in declarative memory or arousal/vigilance. Suggests working memory is more sensitive to acute effects of steroids than declarative memory. Study has not been replicated.
EVIDENCE SUPPORTING : Age by duration effects of steroids on declarative memory			
Keenan et al., 1995	Young adults (< 45 years) and Older adults (46-72 years)	Prednisone - longterm treatment	Found that the detrimental effects of prednisone appear to plateau by the first three years of treatment. This age by duration effect was only identified in the older adult group. However, these results have not been replicated.
EVIDENCE SUPPORTING : The effects of steroids on circadian variation			
Fehm-Wolfsdorf et al., 1993	Young, healthy adults	Hydrocortisone – 50mg	Found that the administration of steroids suppressed the effects of circadian variation. Treatment had no effects on free recall performance, i.e., no significant difference between morning and afternoon performance levels. Performance of control group was higher in morning than afternoon

1.3.6. Summary

Research into the effects of steroids on memory has highlighted several important factors that have been shown to influence these effects. First, the use of different protocols, tasks and steroid-types must be a prime consideration when interpreting the results of different studies. The time of testing relative to the administration of steroids can also influence which stage of the long-term memory process is affected (i.e., working memory or declarative memory). Consequently, the cognitive tests used to measure these effects must be administered at the appropriate time to identify at which stage the detrimental effects of cortisol occur (i.e., during encoding, consolidation and/or during retrieval). There are also the additional effects of age of participant, and dosage and duration of treatment with steroids. Finally, the administration of steroids eliminates the effects of circadian variation which, in turn, may also modify any other time of day effects on memory. This latter result is probably the most significant difference between endogenous and exogenous corticosteroids.

1.4 Overall Summary

As described earlier, our current understanding of the effects of cortisol is far from complete. Indeed, whilst a review of the literature has identified several factors which can modify the effects of cortisol on memory, there are others which remain largely unexplored. One such factor is time of day. Apart from the one study by Fehm-Wolfsdorf et al. (1993) time of day effects have generally been controlled for in previous studies, with researchers testing participants at the same times of day (e.g.,

Kirschbaum et al., 1996). Research into the time of day effects could provide further information regarding the effects of arousal and the inverted U-shaped relationship between cortisol and memory, and the nature and magnitude of effects produced. For example, there may be times when an increase in cortisol levels can be beneficial, or when the administration of steroids is less harmful.

The inverted U-shaped relationship between cortisol and memory, and the degree and magnitude of the effects produced, also suggests there is an optimum level at which cortisol may enhance memory performance. However, the focus of previous research has tended to be on the effects of increasing levels of cortisol, with no investigations on the effects of no- or minimum-levels of cortisol. With evidence to suggest that acute periods of controllable stress (e.g., eustress) can be beneficial, further investigations need to be carried out to identify when and what these 'beneficial' levels may be.

Compared to the effects of chronic changes in cortisol levels, the effects of acute changes in cortisol levels are less clear. Indeed, apart from the recent study by Lupien et al. (1999) no studies have investigated the effects of acute changes in cortisol levels on working memory. This study needs to be replicated to identify whether working memory is, indeed, more sensitive to the effects of acute changes in cortisol than declarative memory.

Although previous research suggests that the detrimental effects of cortisol are brought about via increased activation of GRs (as opposed to MRs), there appears to be no previous research in humans to support this. Activating the two corticosteroid receptors using different types of steroids will help determine whether the harmful effects on memory are brought about via increased activation of the GRs and, if so,

this may have implications for the types of steroids administered during steroid therapy.

The experiments described in this thesis were designed to investigate these issues. Experiment 2 was designed to investigate the additional effects of time of day and acute changes in cortisol levels (both high and low) on working memory and the episodic and semantic components of declarative memory in healthy young males. These were examined under three different conditions of cortisol levels (i.e., high levels, normal daily levels and low levels) and manipulated at each of two different times of day. In contrast, Experiment 3 was designed to investigate the effects on memory produced following activation of the different corticosteroid receptors. In this study, the effects of chronic changes in cortisol levels were investigated in a group of patients with Addison's disease. These effects were examined following acute activation of: the MRs only; GRs only; and both types of corticosteroid receptors, at the same one time of day. Experiments 1 and 2 are now described in Chapters 2 and 3 respectively.

2. The Dose-range Studies

2.1 Abstract

Doses of hydrocortisone (a commonly used steroid) and metyrapone (an 11-β-hydroxylase inhibitor of cortisol synthesis) were administered to healthy young males to identify the doses required to produce different levels of the stress hormone cortisol. In the hydrocortisone study, four male participants were instructed to self-administer four different 'test' doses of hydrocortisone tablets, two in the morning (20 mg and 10 mg) and two in the afternoon (10 mg and 15 mg). The doses administered were at levels designed to produce acute moderate stress levels of cortisol in the morning and 'normal' morning cortisol levels in the afternoon. Levels of cortisol were determined from both serum and saliva samples. Serum samples showed that 30 mg of hydrocortisone administered over two hours in the morning (07.00 and 08.00 hrs respectively) produced moderate stress levels of cortisol at 09.00 hrs (approximately 1000 nMols/L), and 10 mg hydrocortisone administered over three hours in the afternoon (14.00, 15.00 and 16.00 hrs respectively) produced morning levels of cortisol at 17.00 hrs (between 450-700 nMols/L).

In the metyrapone study, one male participant was instructed to self-administer two different 'test' doses of metyrapone tablets on two separate days (750 mg and 1500 mg). The doses administered at 10.00 hrs were designed to reduce endogenous cortisol levels to a minimum two hours later (approximately 150 nMols/L). Levels of cortisol were determined from serum samples only, which showed that 1500 mg of metyrapone administered at 10.00 hrs in the morning reduced cortisol levels to minimum levels by 12.00 hrs (160 nMols/L).

2.2 Introduction

As described at the end of Chapter 1, the purpose of Experiment 1 was to investigate the effects of three different acute changes in cortisol levels and time of day on working memory and declarative memory performance. The three conditions under which memory was tested were the three levels of cortisol. These were increased cortisol levels (in the high cortisol condition); normal daily cortisol levels (in the control condition); and reduced cortisol levels (in the low cortisol condition).

The cortisol levels in the high and low cortisol conditions were manipulated using medication. These comprised hydrocortisone (a steroid) to increase cortisol levels and metyrapone (which inhibits the release of endogenous cortisol) to reduce cortisol levels. One of the reasons for using medication to manipulate cortisol levels was to control for the problems of finding a reliable stressor. The second reason was to control for the potential problems associated with individual differences in the cortisol-response to a stressor identified in previous research. The purpose of Chapter 2, therefore, is to describe the two dose-range studies that were carried out to identify the doses of medication which were administered. Details of some of the evidence reporting individual differences in cortisol-response are also discussed.

2.2.1. Individual differences in cortisol response

Chapter 1 described how cortisol levels are widely regarded as an objective index of psychological stress, more specifically, that increased stress levels are normally associated with the increased release of cortisol (Kirschbaum, Pirke et al., 1995). However, according to Kirschbaum, Diedrich, Gehrke et al. (1992), psychological stress is only accompanied by the release of cortisol if

the individual perceives the event as stressful. Consequently, this suggests that the ways in which individuals respond to stressors can also influence the effects of cortisol on their memory performance.

Chapter 1 also described how there are individual differences in the cortisol response. In a series of different studies, Bohnen et al. (1990), Kirschbaum et al. (1996) and Lupien et al. (1999) classified participants as being either high responders or low responders based on their cortisol responses to experimentally induced stress. Each group of researchers found that the high responders performed significantly worse than the low responders in declarative memory did, however, their classifications of high and low responders were slightly different. All of the researchers classified the high responders as those individuals who responded earliest to the stressor with an increase in cortisol levels. However, Kirschbaum et al. classified high responders as those whose cortisol levels were significantly lower five minutes before the administration of the stressor as opposed to after it, whereas Lupien et al. and Bohnen et al. classified high responders as those who produced higher cortisol levels sixty minutes prior to the stressor as opposed to twenty-five minutes beforehand. More fundamentally, however, whereas Kirschbaum et al. associated the detrimental effects on declarative memory with the increase in cortisol levels produced in response to the stressor per se., both Lupien et al. and Bohnen et al. associated the detrimental effects with the increase produced in anticipation of the stressor.

In contrast to these studies, Smyth et al. (1998) found that it was both the actual experience of the stressor and the anticipation of it that was associated with increased salivary cortisol levels. In this study, participants

were beeped (using a pre-programmed wristwatch) twelve times a day between 08.00 hrs and 21.00 hrs. The beeps were randomly distributed to make it difficult for participants to anticipate the exact time they would occur. During six of the beeps, participants were asked to note down: their activity at the moment of the beep; their location; the presence of others; their affect; and the occurrence of acute stressors (i.e., naturally occurring stressful situations). For the other six beeps, they had to provide a saliva sample. Smyth et al. found that, although average increases in cortisol were relatively low, inter-individual variability in response did exist. They also identified a negative correlation between mood and cortisol levels (i.e., negative affect was associated with higher cortisol levels, and vice versa). More importantly, however, when affect was controlled for, daily stressors were not predictors of cortisol secretion. This highlights the importance of considering an individual's mood state when measuring the effects of stressors on cortisol-response.

Brown et al. (1996) looked at the relationship between individual differences in repressive-defensiveness and basal salivary cortisol levels to see if an individual's coping style might be a predictor of their cortisol response. They found that repressors and high-anxious participants demonstrated higher basal cortisol levels than low-anxious participants did. Indeed, they suggested that both heightened distress and the inhibition of distress might be independently linked to relative elevations in cortisol.

- *Genetic, environmental and personality factors*

Factors such as genetic vulnerability, previous stress experience, coping and personality styles, have also been identified as potential determinants of cortisol response and HPA dysregulation (Heim, Ehlert & Hellhammer, 2000). For example, Wuest, Federenko, Hellhammer & Kirschbaum (2000) measured the levels of salivary cortisol produced at 0, 30, 45 and 60 minutes after wakening in 52 monozygotic and 52 dizycotic twin pairs. They also obtained samples at 08.00 hrs, 11.00 hrs, 15.00 hrs and 20.00 hrs to investigate the short daytime profile. Whilst Wuest et al. found no genetic influence on the short daytime profile, they did identify a significant impact of genetic factors on levels of cortisol produced during the awakening response. They also identified a significant association between several psychological variables (i.e., perceived chronic stress, social stress and lack of social recognition) with the awakening cortisol response.

Levels of self-efficacy and self-esteem have also been shown to have a significant effect on cortisol response (Schaubroeck, Jones & Xie, 2001; Pruessner, Hellhammer & Kirschbaum, 1999). Scaubroeck et al. found that having high self-efficacy lessened the link between having high job demand and poor health; having high job demand exacerbated this effect in individuals with low self-efficacy. Pruessner et al. identified a negative relationship between free cortisol response to a stressor (i.e., a time-pressured arithmetic task) and levels of self-esteem. They only observed this during the difficult task, however, which had been designed for participants to fail; no such effect was observed during the easier task, which was designed for participants to achieve.

Notwithstanding this, however, evidence relating to the effects of individual personality styles on cortisol response has been mixed. For example, Schommer, Kudielka, Hellhammer & Kirschbaum (1999) did not identify any relationship between personality traits (as measured by the Eysenck Personality Questionnaire) and either baseline or stimulated levels of cortisol. Indeed, the cortisol responses produced in response to the single exposure of psychosocial stress, as well as circadian salivary-free cortisol patterns, did not distinguish between participants with high or low scores on extraversion, neuroticism or psychoticism, respectively. Kirschbaum et al. (1993) also found that, whilst the results of their study using the TSST suggest that gender, genetics and nicotine consumption can influence an individual's cortisol-response to psychological stress, there was no correlation with personality traits.

- *Gender differences*

Whilst women may not actually experience more stressful life events or consider specific life events to be more stressful than men, it has been shown that at similar levels of stress, women report significantly greater intensities of symptoms (Wofford, Daly & Juban, 1999). This greater intensity of stress symptoms, however, is not necessarily reflected by their cortisol-response. For example, Kirschbaum, Wuest & Hellhammer (1992) found that the mean cortisol responses produced by men in response to carrying out the TSST were between 1.5- to 2-fold higher compared to women of the same age (i.e., aged 15-33 years). They also found that the men showed elevated cortisol levels in anticipation of the stress without actually having to perform the task; the

women showed no change in cortisol levels during this time. According to Lupien et al. (1997) it is the anticipation of stress that causes the detrimental effects on declarative memory. Similar results were also identified by Kirschbaum et al. (1996) who found that the increase in cortisol levels was more pronounced in the men compared to the women following the TSST.

One explanation for why women may show greater intensities of stress symptoms is that women may be more willing than men to admit to symptoms. Alternatively, men may express their symptoms in different ways (e.g., through alcohol abuse or aggressive acts). Gender differences in the way stress is manifested within an individual's personality were identified by Wofford et al. (1999). They found that the ways individuals manifest their reaction to stress included anger-irritability, negative self-esteem, locus of control and negative affectivity. They also found that, in a mixed group of college students, the females manifested stress more psychologically (e.g., by negative self-esteem and locus of control), whereas the males manifested stress more affectively (e.g., by anger-irritability and negative moods).

Gender differences in response to stress may also be associated with differences in physiological response to stress. For example, women's' blood pressure goes up less than men's' in reaction to stress, although their response increases noticeably after menopause or hysterectomy; this suggests a buffering effect from oestrogen (Stoney, 1999). Kirschbaum, Kudielka, Gaab, Schommer & Hellhammer (1999) recently identified a relationship between oestrogen and corticosteroids. They found that different levels of oestrogen, as produced during menstruation and by oral contraceptives, can exert important effects on HPA responsiveness to psychosocial stress. They also suggested

that, although men tend to show a stronger hypothalamic drive in response to stress, sex differences in cortisol-free levels may be explained by estradiol-induced changes in corticosteroid-binding protein levels. For example, Kirschbaum et al. found that females during the luteal phase of their menstrual cycles showed the greatest salivary cortisol response pattern to the TSST. This response was greater than that shown by the males, and by the females using oral contraceptives and during the follicular phase of their cycles. Similarly, in an earlier study they found that females using oral contraceptives, in comparison to non-users, produced significantly higher cortisol responses to a psychological stressor; there were no significant differences between the two groups in baseline cortisol levels (Kirschbaum, Pirke et al., 1995).

Seeman et al. (1997) investigated the relationship between cortisol secretion and memory performance in men and women and found that high cortisol secretion in women was associated with poorer baseline memory performance. These results were independent of psychosocial variables, including social network ties, frequency of emotional and instrumental support from these ties, measures of self-efficacy beliefs, and depressive symptomatology. No significant associations were found between these measures amongst the men. Seeman et al. also found that the women who exhibited increases in cortisol secretion over the 2.5 year period since baseline, were more likely to show declines in memory performance. In addition, the women who had experienced declines in cortisol levels since baseline, exhibited memory improvements (i.e., the detrimental effects on memory were reversed when their cortisol levels decreased). Evidence for the influence of gender on cortisol-response, however, has been mixed. Indeed, Naber, Sand &

Breitinger (1996) found no differences between males and females in cortisol-response.

- , *Individual differences in habituation to stress*

As described earlier, although cortisol levels may increase in response to stress, how the individual perceives the stressor and copes with it is also important. Indeed, individuals can become habituated, or 'toughened', to recurrent stressors (Epel et al., 1998) and individual differences in habituation to stress have also been identified. For example, Gerra et al. (2001) exposed a group of twenty healthy young males to the same psychosocial stressor (i.e., the Stroop Colour Word Interference task and a public speaking and mental arithmetic task in front of an audience) on two separate occasions (i.e., on days 1 and 8 respectively). After stress exposure on day 1, they found that the plasma concentrations of cortisol (amongst other neuroendocrine responses) in all the participants were significantly elevated. After exposure on day 8, however, a cluster analysis revealed two groups of participants, each showing different habituation patterns for cortisol. On day 8, twelve participants showed a reduction in plasma concentrations of cortisol, whereas eight participants showed a significant increase in plasma concentrations of cortisol. This suggests individual differences in habituation to stress that, in turn, may influence the effects of corticosteroids on memory, as well as on mood regulation and health.

2.2.2. *Rationale behind dose-range studies*

In conclusion, therefore, whilst the results examining the effects of personality and gender on cortisol-response to a stressor are mixed, it is apparent that individual differences can occur. Consequently, these differences need to be considered if the effects of cortisol on memory performance are to be interpreted correctly. Chapter 1 described how the effects of cortisol on memory have been investigated following elevations in both endogenous and exogenous cortisol. It has also shown that the effects on memory produced by exogenous steroids, although different in significant aspects (e.g., effects on circadian variation), are similar to those produced following elevations in endogenous cortisol (Deptula, 1983; Keenan et al., 1995, 1996). Consequently, in order to control for individual differences in cortisol-response and the problems associated with finding a reliable stressor, it was decided that the cortisol levels for the high and low cortisol conditions in Experiment 1 should be manipulated pharmacologically. In this way, although individual differences in the effects of cortisol on memory performance may still be found, at least the levels of cortisol produced prior to testing, would be controlled for (i.e., high for the high cortisol condition and low for the low cortisol condition).

Two dose-range studies were carried out. The first dose-range study involved hydrocortisone, to produce moderate stress levels of cortisol at 09.00 hrs and morning levels at 17.00 hrs. The second dose-range study involved metyrapone, to reduce cortisol levels to minimum levels at both times of the day. The hydrocortisone dose-range study is described first.

2.3. Hydrocortisone dose-range study

Hydrocortisone is a commonly used steroid that increases cortisol levels. It activates both types of corticosteroid receptors (i.e., MRs and GRs) and penetrates the brain rapidly (Lupien & McEwen, 1997). Consequently, this means hydrocortisone is an appropriate type of steroid for measuring the immediate effects of acute changes in cortisol levels on memory performance (i.e., a period of delay is not required between administration and testing to give the steroid sufficient time to penetrate the brain).

As described in Chapter 1, endogenous plasma cortisol levels in healthy adults are normally high in the morning (i.e., approximately 700 nMols/L after arousal) and low in the evening (i.e., less than 10 nMols/L around the hour after midnight; Keenan & Kuhn, 1999). In adults experiencing moderate stress, these peak levels can increase to more than 1000 nMols/L in plasma (Keenan & Kuhn, 1999). As the administration of steroids blocks the effects of circadian variation (Fehm-Wolfsdorf et al., 1993), as well as the production of significant levels of endogenous cortisol (at least temporarily, from 10 to 30 days; Huppertz & Pfuller, 1997) it could be predicted that a healthy individual who receives the same dose of steroids throughout the day will, potentially, have levels of cortisol that remain the same.

One of the aims of Experiment 1 was to investigate the effects of different acute changes in cortisol levels and the additional effects of time of day (e.g., arousal levels) on declarative and working memory. The reason for this was to further investigate the inverted U-shaped relationship between corticosteroids and memory performance, and the direction and magnitude of the effects produced. It has been suggested that the administration of different doses of steroids at different times of day might reveal valuable information regarding this inverted U-shaped relationship (Lupien & McEwen, 1997). Consequently, the purpose of the hydrocortisone dose-

range study was to identify the doses which, when administered orally at 07.00 hrs and 15.00 hrs (on separate days) would, respectively, increase cortisol levels to those produced by moderate stress in the morning (i.e., approximately 1000 nMols/L in plasma at 09.00 hrs) and morning levels in the afternoon (i.e., between 450-700 nMols/L in plasma at 17.00 hrs).

Previous researchers looking at the effects of cortisol on memory have used various doses of hydrocortisone. For example, Beckwith et al. (1986) administered 5, 10, 20 and 40 mg of hydrocortisone, Kirschbaum et al. (1996) administered 10 mg. and Fehm-Wolfsdorf et al. (1993) administered 50 mg. It was, therefore, predicted that a total of either 20 mg or 30 mg of oral hydrocortisone, administered in split doses at 07.00 and 08.00 hrs, would produce moderate stress levels of cortisol at 09.00 hrs. It was also predicted that a total of either 10 mg or 15 mg of oral hydrocortisone, administered in split doses at 14.00 hrs, 15.00 hrs and 16.00 hrs, would produce morning levels of cortisol at 17.00 hrs. (The administration of doses was split to give the students a normal diurnal rhythm and have the appropriate levels wanted at the appropriate time of day.) Consequently, the doses of hydrocortisone administered in the dose-range study comprised a total of either 20 mg or 30 mg in the morning, and either 10 mg or 15 mg in the afternoon. The morning doses were administered on two separate days, at 07.00 hrs and 08.00 hrs (i.e., 15 mg and 5 mg, respectively, in Condition 1, and 20 mg and 10 mg, respectively, in Condition 2). The afternoon doses were also administered on two separate days, at 14.00 hrs, 15.00 hrs and 16.00 hrs (i.e., 5 mg, 2.5 mg and 2.5 mg in Condition 3, and 7.5 mg, 5 mg and 2.5 mg in Condition 4). The levels of cortisol produced by the different doses of hydrocortisone were tested one hour following administration of the final tablet (i.e., at 09.00 hrs for the morning doses and at 17.00 hrs for the afternoon doses). These were tested using

samples of serum and saliva that were obtained from participants at the Clinical Investigation Unit, Bristol Royal Infirmary.

2.4. Methods for hydrocortisone dose-range study

2.4.1. Participants

Four participants (mean age 29.5 years) were recruited into the dose-range study. Recruitment was made by adverts placed in the departments of Experimental Psychology and Medicine, University of Bristol, and by the Psychology Department email system. All participants were males to control for gender differences in the cortisol response (Kirschbaum et al., 1993; 1995). They were also young and healthy to control for the confounding effects of age and pathology (Lupien et al., 1997; Mauri et al., 1993). In addition, all participants were non-smokers and medication-free, and were asked to refrain from consuming both alcohol and recreational-drugs for up to 24 hours prior to testing.

2.4.2. Measures of cortisol

- *Serum cortisol*

Blood samples were taken one hour following administration of the final tablet of hydrocortisone, on each of the four testing days (i.e., two at 09.00 hrs and two at 17.00 hrs). Each participant took part in each condition which meant that a total of four samples were taken from each participant. As mentioned previously, a qualified nurse in the Clinical Investigation Unit obtained all samples using BD Vacutainer plain tubes with gel (i.e., there was no

preservative in the tubes). Once obtained, the samples were then left to clot for 30 minutes and spun in a centrifuge for 15 minutes, at 3000 rpm, 4°C. The liquid on top of a sample after spinning is called the supernatant. The supernatant was pipetted into labelled eppendorfs and stored in a freezer at the Bristol Royal Infirmary laboratories as preparation for analysis.

Serum cortisol levels were determined on an automated immunoassay analyser (ACS : 180 - Chiron Diagnostics Ltd, Colchester Road, Halstead, Essex, CO9 2DX). The detection system is chemiluminescence. Cortisol is a competitive assay with cortisol labelled with acridinium ester. The cortisol antibody is bound to magnetic particles forming the basis of the separation step. With- and between-run coefficient of variations (CV's) are 4.5-7.6 and 6.4-9.7% (i.e., inter-assay = 10-15%; intra-assay = 5%), over a concentration range of 80 – 1000 nMols/L. Working range is 5-2000 nMols/L. Sample volume was 20 microlitres.

- *Salivary cortisol*

As for the serum cortisol, all participants were asked to provide one saliva sample one hour after taking the final tablet of hydrocortisone on each of the four testing days (i.e., a total of four saliva samples were taken for each participant). All saliva samples were obtained using salivettes (Sarstedt), which were given to participants by the researcher upon arrival at the Clinical Investigation Unit.

The procedure for using salivettes is simple. Each salivette contains a cotton wool swab, suspended in an inner tube, which the participant must extract by removing the tube stopper. The swab must be chewed for 1-2

minutes or until fully saturated. The cotton wool swab is then returned back to the inner tube and the stopper is replaced. The salivettes can then either be stored for a couple of days in the fridge (or frozen longer term), as part of the preparation for analysis. In this dose-range study, all samples were frozen and stored in the Bristol Royal Infirmary laboratory, without spinning, in preparation for later analysis.

In comparison to plasma cortisol, salivary cortisol is a measure of the level of free cortisol (i.e., the active unbound cortisol) in the blood. However, as salivary cortisol correlates well with plasma cortisol (Levine, Beattie, McLean & Corman, 1987; Kirschbaum & Hellhammer, 1994) and can be obtained without the confounding effects of venepuncture-induced stress (Vedhara et al., 1999), it was decided that salivary cortisol only would be measured during the main student study. As an added precaution, however, both salivary and serum levels of cortisol were obtained during the dose-range study.

To maintain consistency, all saliva samples were analysed at the same time using the same assay, as follows. All samples were frozen at -20°C upon arrival in the laboratory. Once thawed, the saliva samples were spun at 3000 rpm for 15 minutes. The radioimmunoassay was performed in a 96 well plate. A volume of 25 μL of samples was added to wells which contained 75 μL buffer (0.02 mM sodium citrate, 0.049 mM sodium dihydrogen orthophosphate dihidrate and 0.1% bovine serum albumin), pH 7.2-7.4, 50 μL antibody (Bioclin Cortisol-3-OCMO, Bioclin, Cardiff, UK), and 4000-5000 cpm iodine-125 cortisol (Amersham, UK). All samples were run in duplicate. The plates were then mixed and left to incubate overnight at 4°C. A volume

of 100 μL of charcoal was then added to each well before spinning at 3000 rpm for 15 min. Equal volumes of sample and Optiphase Supermix (Fisher Chemicals, Loughborough, UK) were placed into Wallac plates and counted in a liquid scintillation counter (Wallac Oy, Turku, Finland). Results were calculated from a standard curve.

2.4.3. Other Measures

- *Perceived levels of stress*

It has been suggested that the way in which individuals perceive a stressor can influence the levels of cortisol produced (e.g., Kirschbaum et al., 1992). For example, Van Eck & Nicholson (1994) investigated the relationship between perceived stress levels and cortisol levels using the Perceived Stress Scale (Cohen, Kamarck & Mermelstein, 1983). They found that on workdays, there was a significant positive correlation between perceived stress scores and levels of salivary cortisol. Lupien et al. (1998) also found that participants with increasing/high cortisol reported feeling higher levels of stress over a 30-day testing period. Alternatively, Malarkey et al. (1995) found that it was only the students who perceived the most stress prior to examinations that produced significantly increased cortisol levels.

The evidence for a relationship between perceived levels of stress and cortisol-response, however, is mixed. For example, De Quervain et al. (2000) found that one hour following the administration of 25 mg hydrocortisone, participants did not report any increase in perceived stress levels. Van Eck, Nicholson, Berkhof & Sulon (1996) also found no relationship between the perceived stress levels of a group of white collar males and their cortisol

responses to a stress-inducing speech task. In addition, during exams Malarkey et al. (1995) only identified a relationship between perceived stress levels and daytime cortisol levels in the group of students whose perceived stress scores increased from baseline. This lack of relationship has been explained by the fact that elevated cortisol is a consequence rather than a cause of stress (De Quervain et al., 2000).

In a more recent study, Vedhara et al. (2000) identified a dissociation between perceived stress levels and levels of cortisol. They identified an increase in perceived stress levels during an exam period, but found that this was associated with a significant reduction in salivary cortisol levels. In addition, this reduction in cortisol levels was associated with enhanced short-term memory (as measured by the number of words recalled in a free-recall task), but with no significant effects on auditory working memory.

As part of this dose-range study, participants were asked to report their current perceived levels of stress prior to each testing session (i.e., at 09.00 and 17.00 hrs respectively). This was measured using a self-report Likert rating scale, ranging from 0 (for no stress) to 10 (for high stress), to see if there was any relationship between either serum and/or salivary cortisol and state levels (i.e., levels at the time of completion) of perceived stress.

- *Dietary intake and caffeine consumption*

Prior to each testing session, participants were also asked to report any items of food, including those containing caffeine, they had consumed during the day prior to testing. As part of the testing regime, participants were instructed to eat their 'normal' meals (i.e., breakfast and lunch) on each of the testing days,

but to refrain from eating anything two hours prior to testing. This was to allow glucose levels to stabilise.

Previous research has identified an association between cortisol levels and category of food (i.e., proteins vs. carbohydrates). For example, an increase in salivary cortisol, proportional to the level of protein consumed, was found after a high- but not low-protein meal (Gibson et al., 1999). High carbohydrate diets have also been associated with an increase in testosterone and decrease in cortisol levels when compared with high protein diets (Anderson, Rosner & Khan, 1987). An association between cortisol levels and caffeine levels has also been found. For example, a recent study has shown that caffeine alone can elevate cortisol levels, with more immediate responses presenting themselves in high risk individuals (e.g., hypertensives and high stress responders) compared to low risk individuals (Al Absi et al., 1998).

One way to control for caffeine effects is to ask participants to refrain from consuming any items containing caffeine. However, although caffeine withdrawal has shown few effects on performance levels (Rogers & Richardson, 1995, b), the potential for increased negative mood following withdrawal may be detrimental. As such, all participants were asked to report their caffeine intake for the 24 hours prior to testing, using a checklist (see Appendix I). Food-type and caffeine levels were then recorded in case there was a need to use them as covariates during the analysis of the results.

2.4.4. *Procedure for testing*

Prior to assessment, all participants were given pre-printed instructions on how to prepare for each testing session (see Appendix II). As part of these

instructions, participants were again informed about the purpose of the study and given an overview of what they would be asked to do. This included full details regarding dates and times for administration of the tablets, the criteria they would have to adhere to and specific instructions relating to each of the four testing days.

Each testing regime varied according to the times of testing (i.e., 09.00 hrs or 17.00 hrs) and dose of hydrocortisone administered (i.e., 10 mg, 15 mg, 20 mg or 30 mg). See Table IV for full details. Participants were also asked to record their approximate caffeine-and food-intake as specified. They were also asked to remain alcohol and recreational drug free for up to 24 hours prior to testing, and were made aware that they would be asked to consume hydrocortisone and provide a venepuncture sample of blood. Written informed consent was obtained (see Appendix III).

All participants were asked to take the first tablets, with food, and not to eat anything for two hours prior to testing. They were informed that this was to allow glucose levels to stabilise.

Table IV : Dose of hydrocortisone and time of day of self-administration

Condition	Time of Day of administration	Time of Day of testing	Dose of Hydrocortisone
1	07.00 hrs 08.00 hrs	09.00 hrs	15.0 mg 5.0 mg
2	07.00 hrs 08.00 hrs	09.00 hrs	20.0 mg 10.0 mg
3	14.00 hrs 15.00 hrs 16.00 hrs	17.00 hrs	5.0 mg 2.5 mg 2.5 mg
4	14.00 hrs 15.00 hrs 16.00 hrs	17.00 hrs	7.5 mg 5.0 mg 2.5 mg

Once the blood and saliva samples had been obtained, participants were told to wait at least fifteen minutes before departure to ensure that any effects produced by the venepuncture (e.g., feeling faint) did not occur. A minimum period of one week was also allowed between each testing session. This was to enable any change in endogenous cortisol levels brought about following the administration of hydrocortisone to return to normal.

2.5. Results of hydrocortisone study

The three primary variables in this study were dose of hydrocortisone and levels of salivary and serum cortisol. In addition, the effects of a number of secondary variables were also explored. The relationships between these variables were examined using a series of Pearson's Product Moment correlations shown in Table V.

Table V : Showing the relationships between total dose of hydrocortisone administered (i.e., 75 mg), serum cortisol, salivary cortisol, perceived stress levels and age.

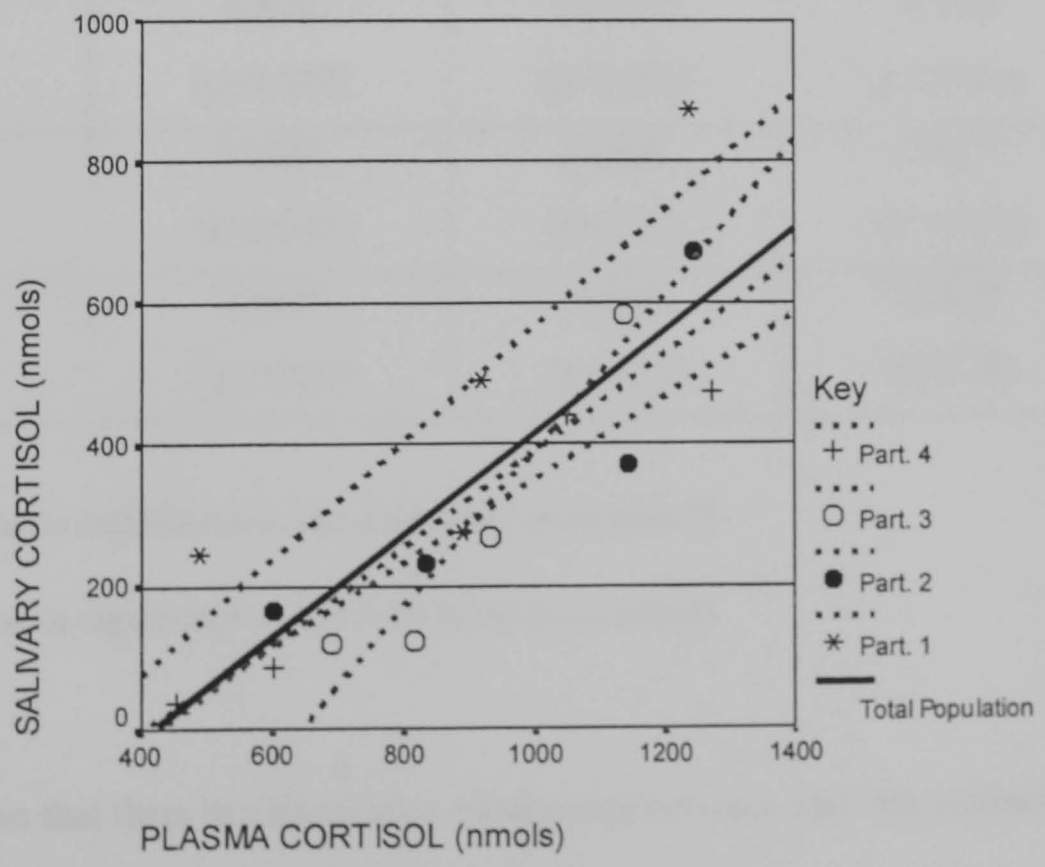
	DOSE	SERUM	SALIVA	STRESS	AGE
DOSE	1.000 .				
SERUM	**.922**** **.000**	1.000 .			
SALIVA	**.881**** **.000**	**.837**** **.000**	1.000 .		
STRESS	-.050 NS	-.025 NS	-.186 NS	1.000 .	
AGE	.000 1.000	-.136 NS	-.191 NS	.303 NS	1.000 .

** Correlation is significant at the .01 level (2-tailed)

The results show that, whilst there are significant relationships between dose of hydrocortisone, serum cortisol and salivary cortisol levels, there are no significant relationships between the primary variables with age or perceived levels of stress.

A statistical assumption when calculating correlations is that the data are all independent. However, in this instance each participant contributes four data points, relating to the dose of hydrocortisone administered and time of day of testing (i.e., 30 mg or 20 mg for testing at 09.00 hrs, and 15 mg or 10 mg for testing at 17.00 hrs). There are several alternative and complimentary approaches for dealing with this assumption (Wright, 1998), the simplest being to run separate linear regressions for each participant. Graphically this is presented in Figure 3 as separate regression lines for each participant, which compare the different stress measures with, first each other, and then with dose.

Figure 3 : Showing relationships between (1) plasma (serum) and salivary cortisol levels for each individual and (2) group mean plasma (serum) and salivary cortisol levels.



The graph highlights the significant relationship between the mean serum and salivary cortisol levels for the group. It also shows that the relationships between serum and salivary cortisol for each of the four individuals are similar. Consequently, this implies that, even with a small sample of only four participants, the results produced can be considered reliable.

A series of Pearson's Product Moment correlations were also carried out looking at the relationship between the total levels of salivary and serum cortisol produced, and total dose of hydrocortisone administered for each participant. The results are shown in Table VI.

Table VI : Showing Pearson's Product Moment correlations for each participant for relationships between salivary cortisol, serum cortisol and total dose of hydrocortisone.

Participant No	Pearson's r for saliva by serum	Pearson's r for saliva by dose	Pearson's r for serum by dose
1	0.867 (p=0.133)	0.978* (p=0.022)	0.949 (p=0.51)
2	0.893 (p=0.107)	0.991** (p=0.009)	0.936 (p=0.064)
3	0.953* (p=0.047)	0.966* (p=0.34)	0.999** (p=0.001)
4	0.980* (p=0.020)	0.889 (p=.111)	0.962* (p=0.38)

** Correlation is significant at the 0.01 level (two-tailed)

* Correlation is significant at the 0.05 level (two-tailed)

This shows that there is a significant relationship between salivary cortisol levels and dose of hydrocortisone administered for Participants 1 – 3, but not for

Participant 4. It also shows significant relationships between salivary cortisol and serum cortisol for Participants 3 and 4, but not for Participants 1 and 2. Indeed, only Participant 3 showed significant relationships for all three primary variables, (although the sizes of correlations were high for all four participants). This suggests that individual differences in cortisol-response occur even when the same doses of steroids are administered. The results in Table V also suggest that these specific individual differences are not explained by differences in age or perceived stress levels. However, as different people can absorb steroids slightly differently (e.g., Brutsche, Brutsche, Munawar et al., 2000) particularly if they have recently had a meal, this might have been predicted.

The mean and standard deviation levels of cortisol produced following administration of the different doses of hydrocortisone are shown in Table VII. This shows that the mean levels fall within the anticipated ranges for moderate stress and morning levels of hormones. It also shows that the levels of cortisol increased in the predicted directions as the dose of hydrocortisone administered increased.

Table VII : Showing a comparison between observed and expected mean cortisol levels with dose of hydrocortisone administered.

Dose (mg)	Expected Serum Cortisol Levels (nMols/L)	Observed Serum Cortisol (n/Mols/L) Mean	SD	Observed Salivary Cortisol (n/Mols/L) Mean	SD
30	1001-1300	1222.00	59.94	650.75	170.44
20	901 – 1000	1009.25	106.37	392.50	95.89
15	701 – 900	784.00	125.36	178.50	88.94
10	450 – 700	557.25	107.76	141.75	86.88

2.6. Metyrapone dose-range study

The second dose-range study involved metyrapone. Metyrapone is a drug which inhibits the synthesis of endogenous cortisol, which it does by preventing the release of further cortisol when levels become low (Young et al., 1997; i.e., it stops the negative-feedback action of cortisol). It acts at the level of the adrenal and, when given short term, its predominant effect is on GR hormones.

Metyrapone is normally used as a treatment for patients suffering from hypercortisolemia (i.e., the over-production of endogenous cortisol, as found in patients with conditions such as Cushing's Syndrome). It has also been used for its antidepressant-like properties for the treatment of patients with major depression (e.g., Young, Lopex, Murphy-Weinberg, et al., 1997; O'Dwyer et al., 1995; Healy, Karkin, Cryan et al., 1999). For example, Young et al. (1997) found that the administration of 1500 mg metyrapone reduced excessive secretion of cortisol in depressed patients. This was administered in two split doses of 750 mg, one at 08.00 hrs and one at 11.30 hrs.

Chapter 1 described some of the physiological effects identified with high levels of corticosteroids (e.g., immunosuppression and obesity). Physiological effects have also been identified with low levels of cortisol. For example, a lack of cortisol can lead to fatigue, allergies and arthritis (e.g., Cleare, Blair, Chambers & Wessely, 2001). However, whilst the effects of low levels of cortisol have been identified from a physiological perspective, no studies appear to have investigated the effects of significantly low levels of cortisol on memory.

In a similar vein, no studies have investigated the effects on memory when corticosteroid receptors are under-activated. Previous research has shown that

activation of the MRs increases long-term potentiation and, thus, activation of the MRs facilitates learning and memory. It also suggests, however, that although increased activation of the GRs can impair memory performance, a degree of GR activation appears to be a pre-requisite for the long-term storage of information (De Quervain et al., 1998). As stress levels of cortisol activate GRs, this implies that a minimum level of cortisol is required for memory. The aim of this second dose-range study, therefore, was to identify the dose of metyrapone that would need to be administered, as one single dose at 10.00 hrs, to reduce endogenous cortisol levels to very low levels within two and up to three hours later. (Memory performance would be tested between this two to three hour period.) Consequently, based on previous research it was predicted that either 750 mg or 1500 mg of metyrapone, administered at 10.00 hrs, would significantly reduce and maintain low levels of cortisol between 12.00 and 13.00 hrs.

2.7. Methods for metyrapone dose-range study

2.7.1. Participants

Only one male participant (aged 34 years) volunteered to take part in the metyrapone dose-range study. This participant was recruited using the same procedure as for the hydrocortisone dose-range study.

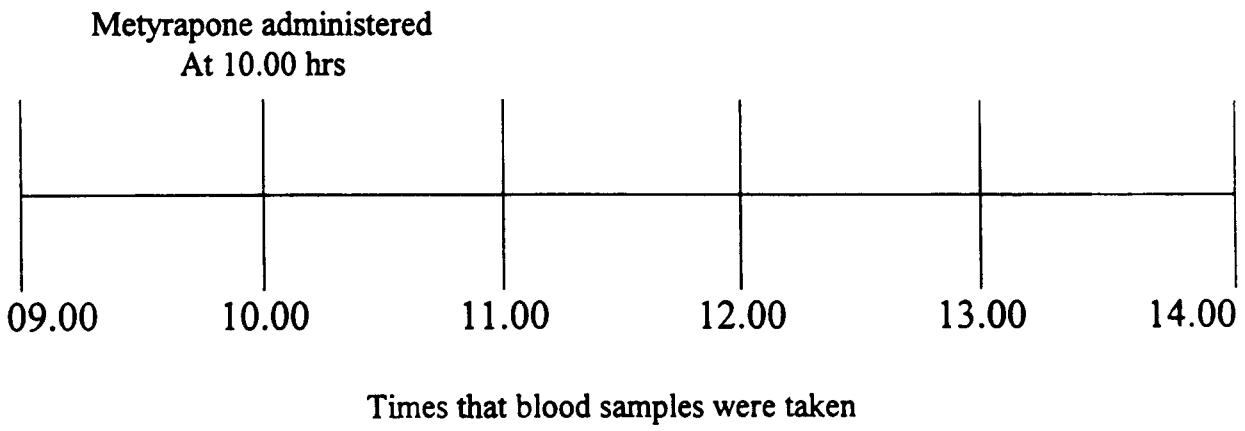
2.7.2. Measure of cortisol

- *Serum cortisol*

The levels of cortisol produced following the administration of metyrapone were measured from blood samples. A total of six blood samples was

obtained for each dose of metyrapone, meaning that a total of twelve blood samples were taken from the same participant. Figure 4 shows the times that each blood sample was taken and the time that the metyrapone was administered for each of the two doses.

Figure 4 : Showing times for metyrapone administration and blood samples.



The metyrapone tablets were self-administered orally and a qualified nurse in the Clinical Investigation Unit, Bristol Royal Infirmary, obtained all blood samples. After each sample was obtained, it was left for 30 minutes to clot and then spun in a megafuge for 15 minutes, at 3000 rpm, 4°C. All samples were then sent to Biochemistry for analysis. Analysis of the serum cortisol was carried out using the same serum cortisol procedure described in the hydrocortisone study.

2.7.3. *Controls*

- *Dietary intake and caffeine consumption*

The measures of dietary intake and caffeine consumption prior to testing were the same as those described in the hydrocortisone dose-range study. The participant was, however, allowed to eat and drink non-alcoholic beverages, as normal, from 09.00 hrs to 14.00 hrs. A note of all items consumed was made.

2.7.4. *Procedure for testing*

A similar testing procedure, as described in the hydrocortisone study, was used. The only differences were the times of testing and the total number of blood samples the participant had to provide (see Table VIII for full details). The same testing regime was used for each one of the two testing sessions; only the dose of metyrapone differed.

As shown in Figure 4, blood samples were taken on each hour from 09.00 hrs to 14.00 hrs inclusive. At each time point the participant was also asked to report how he was feeling, together with what items of food he had eaten during the previous hour.

A period of two weeks was allowed between each testing session. This was to allow any changes in cortisol levels brought about following the administration of metyrapone to return to normal. All testing was carried out in the Clinical Investigation Unit in the Bristol Royal Infirmary, and under the supervision of a qualified nurse.

2.8. Results of metyrapone study

The levels of cortisol measured by each blood sample are shown in Table VIII. The aim of the dose-range study was to identify the dose of metyrapone which, when administered as a single dose, would reduce endogenous cortisol levels to a minimum two hours later (i.e., to between 150 and 200 nMols/L). Table VIII shows that two hours after administration (i.e., by 12.00 hrs), both doses reduced endogenous cortisol levels to within these limits. However, the dose administered also needed to reduce cortisol levels to minimum levels during the one hour of memory testing. This shows that the administration of 1500 mg metyrapone at 10.00 hrs was the only dose to achieve this.

Table VIII : Showing the daily change in levels of cortisol produced following the administration of metyrapone

Dose of metyrapone administered at 10.00 hrs	Time of Day	Levels of Serum Cortisol (nMols/L)
750 mg	09.00 hrs	790[1]
	10.00 hrs	534
	11.00 hrs	277
	12.00 hrs	134
	13.00 hrs	187
	14.00 hrs	293
1500 mg	09.00 hrs	788[1]
	10.00 hrs	588
	11.00 hrs	183
	12.00 hrs	162
	13.00 hrs	129
	14.00 hrs	140

[1] These levels were considered within the predicted range for normal morning cortisol levels

Throughout the dose-range study, the participant did not report any significant effects from the treatment apart from feeling slightly light-headed one hour following the administration of 1500 mg metyrapone. It was, therefore, decided that when administered during the main student study, participants would be instructed not to drive for up to two hours following administration of the 'treatment' on each of their testing days.

2.9. Discussion

The overall purpose of the two dose-range studies described in this chapter was to identify the doses of hydrocortisone and metyrapone that needed to be administered to healthy young males to produce either moderate stress levels of cortisol at 09.00 hrs and morning levels at 17.00 hrs (Condition 1), or minimum levels of cortisol at 09.00 hrs and 17.00 hrs (Condition 2) on different days. As participants were also required to complete one hour of memory tests under each of these conditions, these manipulated cortisol levels needed to be maintained for one further hour.

The results of this current study suggested that the oral administration of 20 mg hydrocortisone at 07.00 hrs, followed by 10 mg at 08.00 hrs produced moderate stress levels of cortisol at 09.00 hrs. Similarly, the oral administration of 5 mg hydrocortisone at 14.00 hrs, followed by 2.5 mg at 15.00 hrs and 2.5 mg at 16.00 hrs, produced 'morning' levels of cortisol at 17.00 hrs. These levels also remained stable for up to one hour. The results also suggest that one single dose of 1500 mg metyrapone, administered at 10.00 hrs, reduces endogenous cortisol levels to a minimum for up to two-three hours later. This dose was also administered safely and without any significant side effects. Consequently, although these latter results were

obtained from one participant only (which was not intended but determined by the lack of availability of willing volunteers), it was agreed that these doses would be suitable for a study designed to investigate the effects of different levels of cortisol on memory performance.

Although body mass index (BMI), and food and caffeine intake details were also recorded as part of this dose-range study, there was no significant relationship between either of these variables with serum cortisol, salivary cortisol or dose of hydrocortisone. In addition, there were no significant effects of either age or perceived stress levels. It is important to note, however, that although the relationships between dose, saliva and serum were very similar for all four participants, a sample size of four is very small. The lack of an apparent relationship between these three main variables with age and perceived stress levels, therefore, was not surprising, especially as the age-range of participants was narrow and cortisol levels were manipulated using medication. Consequently, both age and perceived stress levels were measured in the main study and, potentially, treated as covariates.

Both serum and salivary cortisol levels were obtained during the hydrocortisone dose-range study. However, as these data produced a significant correlation between salivary cortisol and serum cortisol, which is consistent with the results of earlier studies (e.g., Levine et al., 1987), it was decided that only measures of salivary cortisol would be collected in the main study. As mentioned previously, using salivary cortisol controls for venepuncture-associated stress (Vedhara et al., 1999).

In conclusion, therefore, it was decided that participants in the high cortisol condition would receive a total of 30 mg of hydrocortisone in the morning and a total of 10 mg hydrocortisone in the afternoon. Participants in the low cortisol condition

would receive a total of 1500 mg metyrapone in the morning and in the afternoon. The doses of medication would be provided to participants in tablet form, together with full and clear instructions on the times when self-administration should take place.

3. Experiment 2 : The student study

3.1 Abstract

Previous research suggests that declarative memory is sensitive to the effects of chronic changes in cortisol levels, whereas working memory may be more sensitive to acute changes. In addition, there is an inverted U-shaped relationship between corticosteroids and memory that influences the direction and magnitude of the effects produced. The immediate effects of three different acute changes in cortisol levels on working memory and the episodic and semantic components of declarative memory were investigated in three groups of young males (mean age 20 years). They were also measured at each of two times of day (i.e., at 09.00/10.00 hrs vs. 17.00 hrs). Although significant between-group differences in cortisol levels were observed, the results failed to demonstrate significant differences in either working or declarative memory as a function of cortisol levels. However, whilst the results also failed to demonstrate significant differences in either aspect of memory performance as a function of time of day, they did identify a significant positive relationship between morning cortisol levels in the control group and two measures of episodic declarative memory in the morning; this suggests that, in the morning, these aspects of memory performance were facilitated by higher cortisol levels. They also identified a significant negative relationship between afternoon cortisol levels in the high cortisol group and one measure of semantic declarative memory in the afternoon; this suggests that, in the afternoon, this aspect of memory performance was impaired by higher cortisol. The results also identify several possible explanations that suggest that the effects of cortisol on memory performance do not operate in isolation, and may be dependent on other situational and personality variables.

3.2 Introduction

Chapter 1 described how the effects of cortisol on cognitive function are selective. It also described how these differences in effects may be linked to increased activation of the two types of corticosteroid receptors (i.e., MRs and GRs), which are abundant in the hippocampal and frontal lobe regions of the brain. Declarative memory depends on the integrity of the hippocampus, whereas working memory depends on the integrity of the frontal lobes. Consequently, this suggests that declarative memory and working memory may be sensitive to the effects of changes in cortisol.

Chapter 1 also described how, compared to the effects of chronic elevations in cortisol levels, the effects brought about following acute elevations are less clear. Indeed, at the time of writing this thesis, only one previous study had investigated the effects of acute elevations in cortisol levels on working memory (i.e., Lupien et al., 1999). As described previously, Lupien et al. (1999) found that working memory performance was impaired by acute changes in cortisol levels following the administration of the equivalent of 16.6 mg hydrocortisone. Indeed, they interpreted these results as suggesting that working memory may be more sensitive to the effects of acute changes in cortisol levels than declarative memory due to the specific effects of cortisol during encoding; the corticosteroid receptors located in the prefrontal cortex are activated by encoding (Smith et al., 1998;Ungerleider et al., 1998).

Chapter 1 also described how most of the previous research looking at the effects of cortisol on memory has controlled for time of day. Indeed, apart from the study by Fehm-Wolfsdorf et al. (1993), which tested participants at 09.00 hrs and 18.00 hrs, previous studies have tested the effects of cortisol on memory at one time of day only. Moreover, these times of day have been different across studies. For example, Kirschbaum et al. (1996) carried out testing in the afternoon, Newcomer et

al. (1994; 1999) tested participants at 16.00 hrs, and Lupien et al. (1996) tested participants at 13.30 hrs.

The curvilinear relationship between the effects of cortisol on memory performance and time of day occurs because of the circadian variation in endogenous cortisol release, and this occurs regardless of food intake and sleep/wake cycles. To recap, during the 'awakening cortisol response', free plasma cortisol levels increase two to three fold (Hucklebridge et al., 1999). After waking, plasma cortisol levels peak during the morning (i.e., to about 700 nMol/L at peak) and then fall throughout the day, dropping to low levels in the hour after midnight (i.e., around 10 nMol/L; Keenan & Kuhn, 1999).

The effects of time of day on recall performance, as well as day of testing, were investigated by Testu & Clarisse (1999) in 10-11 year olds. In this study, pupils were instructed to listen to a story and learn fourteen nouns at 09.00 hrs or 15.00 hrs on either a Monday or Thursday. Although there were no effects on immediate recall, they found that pupils recalled more words after a period of delay from the list learned at 09.00 hrs compared to those learned from a list at 15.00 hrs; in line with circadian variation, endogenous cortisol levels would be higher at 09.00 hrs compared to 15.00 hrs. The pupils also recalled more words at 09.00 hrs learned on a Thursday compared to those learned on the Monday. This contrasted with the observation made by Folkard, Monk, Bradbury & Rosenthal (1977) who claimed that the diurnal variations in memory performance were independent of testing day. Consequently, this suggests that the effects of cortisol on memory performance may also be affected by testing day, although at the time of writing this thesis, the observations made by Testu & Clarisse had not been reported elsewhere.

Fehm-Wolfsdorf et al. (1993) also investigated the effects of diurnal variation in declarative memory performance but more specifically following acute changes in cortisol levels. By carrying out testing at 09.00 hrs and 18.00 hrs, they found that participants in the control condition, whose levels of cortisol were higher in the morning compared to the afternoon, showed better declarative memory performance at 09.00 hrs compared to at 18.00 hrs. Taken together, therefore, as time of day of testing has been shown to influence memory performance, the effects of acute changes in cortisol levels on both working memory and declarative memory in the current study were examined at two different times of day.

At this point, however, it is important to note that in some individuals the diurnal variation in cortisol release does not occur (Stone et al., 2001); some individuals have erratic or flattened cortisol curves. Flattened cortisol curves show a lack of variation throughout the day and these have been associated with a range of psychological disorders, including: chronic stress (Rosmond, Dallman & Bjorntop, 1998; Chrousos & Gold, 1998); depression (Deuschle et al., 1997); and post traumatic stress disorder (Yehuda, Teicher, Trestman et al., 1996). Furthermore, a recent study by Sephton, Sapolsky, Kraemer & Spiegel (2001) suggested that patients with advanced breast cancer and flattened cortisol curves (i.e., consistently high or erratic rhythms) are significantly more likely to die earlier than those who show normal rhythms. This highlights one of the reasons why the study of the effects of cortisol is such an important area of research.

3.3 The Current Study

Based on the evidence from previous research, therefore, in addition to further examining the effect of acute changes in cortisol levels on working memory and

declarative memory, the current study was designed with four primary objectives in mind. These were: to investigate the effects of reduced levels of cortisol on memory; to investigate the effects of time of day; to identify whether both the episodic and semantic components of declarative memory are affected by acute changes in cortisol; and to identify the stage during the memory process at which the effects of cortisol occur. In addition, the influences of several other variables on the effects of cortisol produced were also examined. These included: BMI; perceived levels of stress; caffeine intake; glucose levels and type of food group consumed. The reasons for doing this are described below.

3.3.1. Effects of acute changes in cortisol levels

As mentioned previously, compared to the effects of chronic changes in cortisol levels on memory the acute effects remain unclear. Consequently, the first aim of this current study was to further investigate the effects of acute changes in cortisol levels and identify whether working memory is, indeed, more sensitive than declarative memory. If the results did identify higher levels of impairment in working memory with little, or no, impairment in declarative memory, then the current study would be one of the first to lend support to that carried out by Lupien et al. (1999).

3.3.2. Effects of high and low levels of cortisol

As described in Chapter 2, previous research has investigated the effects of increased levels of cortisol on memory. However, there do not appear to be any studies that have looked directly at the effects of significantly reduced levels of cortisol on memory performance. The second aim of this current

study, therefore, was to investigate the effects of reduced levels of cortisol (using metyrapone) in addition to 'normal' and increased (using hydrocortisone) levels of cortisol on memory performance. The inverted U-shaped relationship between corticosteroids and memory has suggested that, if cortisol levels are at the peak of the curve, memory performance will be facilitated. Indeed, an acute increase in cortisol levels from rest to stress has been related to positive attributes, including better performance levels (Ellertsen, Johnsen & Ursin, 1978). If, however, levels are either too high or too low (i.e., down the sides of the curve), memory performance may be impaired.

3.3.3. *Additional effects of time of day*

By testing memory performance at two different times of day, the third aim of the current study was to investigate the time of day effects. To do this, testing was carried out at: (1) either 09.00 hrs (for the high cortisol and control conditions) or at 10.00 hrs (for the low cortisol condition), when endogenous cortisol levels are normally high; and at (2) 17.00 hrs, when endogenous cortisol levels are normally low. (The differences in the two morning times of testing were determined by the amount of time required by the medication to produce the required cortisol levels.) In line with the inverted U-shaped relationship between cortisol and memory performance, it was predicted that an increase in cortisol levels when baseline levels are low (e.g., in the afternoon) would enhance memory performance. Conversely, it was predicted that an increase in cortisol levels when baseline levels are high (e.g., in the morning) would impair memory performance.

3.3.4. *Episodic and semantic components of declarative memory*

By using tasks of episodic memory and semantic memory, the fourth aim of the current study was to identify whether both components of declarative memory are dependent on the hippocampus. To recap, Chapter 1 described the two perspectives put forward to explain how the episodic and semantic components of declarative memory work alongside each other. According to the unitary perspective, all items must pass through episodic memory before reaching semantic memory (e.g., Cohen et al., 1997; Squire & Knowlton, 1995; Squire & Zola, 1996). Consequently, according to this view point both components of declarative memory are dependent on the hippocampus. The dissociated perspective, however, claims that episodic memory is not critical for the formation of semantic memory (Parkin, 1982; Cermak, 1984; Kinsbourne & Wood, 1975). Consequently, according to this view point only episodic memory is dependent on the hippocampus. Therefore, if both episodic and semantic memories are impaired by acute changes in cortisol levels, this lends support to the unitary perspective. However, if only episodic memory is impaired, this lends support to the dissociated perspective. Alternatively, if neither episodic nor semantic memory are impaired but working memory is, this suggests that working memory is more sensitive to acute changes in cortisol levels than declarative memory; this would lend support to the study by Lupien et al. (1999).

3.3.5. *Point of effects of cortisol on declarative memory*

By using tasks of free recall and recognition, the fifth aim of the current study was to identify the stage during the declarative memory process that any

effects of cortisol occurred. Chapter 1 described the three stages of long-term memory and how some studies have identified detrimental effects during: acquisition (e.g., Lupien et al., 1999); consolidation (e.g., Lupien et al., 1995; 1999) and retrieval (e.g., De Quervain et al., 2000). However, very few studies have reported the stage at which the effects of cortisol occurred and, as cortisol can have multiple and often conflicting effects on memory function, it is critical to be able to dissociate the effects on the different memory phases in order to interpret the effects on memory correctly (Lupien & McEwen, 1997). Consequently, if the results of this current study identify low free recall and normal recognition performance, this suggests an impairment in declarative memory at the level of recall. If, however, the results identify low free recall and low recognition performance, this suggests an impairment in declarative memory brought about by incorrect encoding due to an overload of working memory.

3.3.6. Other Variables

- *Body Mass Index (BMI)*

Chapter 2 described the details of two dose-range studies that were carried out to identify the doses of hydrocortisone and metyrapone to be administered to participants in the high and low cortisol conditions respectively. As these same doses of medication were administered to participants irrespective of height and weight, each participant's BMI was calculated using the equation *weight divided by (height [in metres] x 2)*. This was to see if the degree of change in cortisol levels produced either endogenously (i.e., in participants in the control condition) or exogenously (i.e., in participants who received

medication in the high and low cortisol conditions) was influenced by body size. If a significant relationship between the two was found, it could therefore be speculated that this might have additional effects of cortisol on memory performance. For example, the effects brought about by the same dose of medication might be greater in an individual with a smaller BMI compared to one with a larger BMI.

- *Perceived levels of stress*

Chapter 2 also described the mixed results pertaining to the relationship between perceived levels of stress and cortisol levels. For example, van Eck et al. identified a positive relationship between perceived levels of stress and cortisol in their study in 1994. They did not, however, identify any relationship in a later study in 1996. Moreover, Vedhara et al. (2000) found an inverse relationship between perceived levels of stress and cortisol. As for the dose-range study, all participants in the current study were asked to report their perceived levels of stress prior to each testing session. This was done using the same self-report Likert rating described in Chapter 2. The reason for taking this measure was to investigate the relationship between how participants perceived their stress levels compared to those produced by medication (i.e., in the high and low cortisol conditions) or time of day (i.e., in the control condition). No previous studies have compared perceived levels of stress with significantly reduced levels of cortisol; consequently the results produced by participants in the low cortisol condition would be unique.

An investigation into whether the method used to manipulate the levels of cortisol (i.e., endogenous versus exogenous) had any effect on perceived

stress levels was also carried out. The reason for this was to see whether there was a difference in the relationship between perceived levels of stress and cortisol levels which occurred naturally (i.e., in the control condition), compared to those which were manipulated pharmacologically (i.e., in the high and low cortisol conditions). A difference between the two might then have implications for whether it is the individual's perception of stress which influences the change in cortisol levels, or vice versa.

- *Caffeine Intake*

Although there are exceptions (e.g., patients with anxiety disorders), there has been considerable evidence to show that regular, moderate caffeine usage facilitates performance levels, particularly if it is consumed when alertness is low (e.g., first thing in the morning or after lunch). For example, caffeine-related improvements in mood and performance have been identified (Jarvis, 1993), and higher caffeine-users have been found to show better mental functioning (e.g., Smith, Kendrick & Maben, 1993). Alternatively, there have been studies where participants have shown increased arousal levels following caffeine consumption, but without showing any effects on memory (Herz, 1999). Smith, Clark & Gallagher (1999) also reported no differences in working memory performance between young adults who were assigned to either a caffeinated versus a de-caffeinated condition. Large doses of caffeine can also increase anxiety levels (see Lieberman, 1992, for a full review) and as increased anxiety levels have also been associated with increased cortisol levels (Brown et al., 1996), caffeine intake was considered a potential covariate in this study.

All participants were asked to record their approximate levels of caffeine consumed from 24 hours prior to testing, for each of the two testing sessions. They were given checklists detailing the most common items containing caffeine to help them with this, which they were asked to complete and bring along to each testing session. These data were then available for treatment as a covariate if appropriate.

- *Type of food group consumed*

Previous research has shown that an individual's serotonin levels increase following a carbohydrate-rich, protein poor diet (Markus, Panhuysen, Jonkman & Bachman, 1999) and that this increase in serotonin levels appears to be a prerequisite to how well an individual copes with stress (Anisman & Zacharko, 1991; Deakin, 1991; Deakin & Graeff, 1991). Indeed, it has also been shown that the increased availability of brain serotonin in highly stressed individuals produced following a carbohydrate-rich, protein-poor diet improves cognitive performance under controllable laboratory stress (Markus et al., 1999).

The results of dietary studies investigating the relationship between carbohydrate-consumption and improved performance levels, however, have been inconsistent. For example, whilst some researchers have found that carbohydrates can improve performance (Kanarek & Swinney, 1990), others have found that they have no effect at all (Lieberman, Cabellero & Finer, 1986; Lloyd, Rogers & Hedderley, 1996) and even more, that they can impair it (Spring, Maller, Wurtman et al., 1982/83). In addition, a comparison of the effects of food versus no-food on cognition has shown that participants who

had no-food prior to testing showed poorer working memory (Smith et al., 1999).

The type of foods consumed prior to each testing session were not controlled for in this current study. Indeed, as for caffeine intake, it was felt that changing an individual's normal eating pattern (e.g., by asking them to eat a bowl of cereal for breakfast when they would normally not eat anything at all) might have additional confounding effects on performance. Therefore, as a relationship between types of food consumed and salivary cortisol levels has been identified (e.g., Gibson et al., 1999 found that salivary cortisol levels increased in direct proportion to the amount of protein eaten during a meal), which might, in turn, affect levels of memory performance, all participants were asked to report which items of food they had consumed during the day prior to testing. By using these data, the researcher was then able to categorise each participant's food consumption into one of three groups: no food consumed; high protein/low carbohydrates; and low protein/high carbohydrates. These data were then available to investigate whether there was any relationship between food group consumed and memory performance and, if appropriate, to treat these data as covariates.

3.3.7. Hypotheses

Based on the review of previous research, the following predictions were made:

Working memory will be more sensitive to the effects of acute changes in cortisol levels than either/both episodic memory or/and semantic memory. This is based on previous research carried out by Lupien et al. (1999).

Acute changes in cortisol levels will impair memory performance in the morning, but will enhance it in the afternoon. This relates to the inverted U-shaped relationship between corticosteroids and memory performance reported by Lupien & McEwen (1997).

The declarative memory performance of participants in the control condition will be better in the morning compared to the afternoon. This is based on the effects of time of day identified by Fehm-Wolfsdorf et al. (1993).

There will be a positive relationship between cortisol levels and perceived levels of stress. This is based on previous research carried out by Lupien et al., 1998.

3.4 Methods

3.4.1. Design

The principle aim of the current study was to identify, (1) the effects of different levels of cortisol (a stress hormone) on memory performance, and (2) the additional effects of time of day. To do this, a single-blind, mixed (3 x 2) experimental design was used, in which three groups of twenty participants were randomly allocated to one of three between-group and each of two within-group conditions. The dependent variable was memory performance, which was measured using a battery of memory tasks designed to test different aspects of short-term working memory and long-term declarative memory. Other measures obtained included: age; BMI; perceived levels of stress; approximate caffeine intake from 24 hours prior to testing; types of food eaten prior to testing; salivary cortisol levels; and glucose levels.

- *Between-group conditions*

The three between-group conditions comprised: (1) a high cortisol condition (using hydrocortisone to increase cortisol levels); (2) a control condition (using calcium carbonate, which has no effect on cortisol levels); and (3) a low cortisol condition (using metyrapone to reduce cortisol levels). Allocation to the between-group conditions was randomised by the investigator who, during the induction session, asked each participant to withdraw a ticket from a bag. The bag contained a total of sixty tickets. Twenty of these were labelled (1), for the high cortisol condition; (2) for the control condition; and (3) for the low cortisol condition. All withdrawn tickets were destroyed to prevent repeated allocation to the same condition. Participants were not made aware

of which condition they had been allocated to until the debriefing session at the end of testing.

- *Within-group conditions*

There were two within-group conditions related to the two times of testing. These were: (1) at either 09.00 hrs or 10.00 hrs (depending on the type of medication administered); and (2) at 17.00 hrs. A minimum period of three and a maximum period of seven days was set between each of the two testing sessions. This was to allow any changes in endogenous cortisol, resulting from the medication, to return to normal. Only one participant was tested during each testing session. Refer to *Allocation of tasks to each battery* for details of how each participant was allocated to each within-group condition.

3.4.2. Participants

A total of sixty male participants were recruited into the study. With twenty participants in each condition, it was calculated that this would produce statistical power effect sizes of .09, .38 and .78 for small, medium and large effect sizes respectively (Cohen, 1988). These calculations were based on Cohen's effect size index for ANOVA's (Cohen, 1992) of 0.10, 0.25, and 0.40, for small, medium and large effect sizes (see Appendix IV for calculation). In other words, according to the definition provided by Thomas & Krebbs (1997), a study with twenty participants in each of three conditions would produce a 78% 'probability of getting a statistically significant large effect'. Lupien et al. (1999) reported significant effects of acute changes in cortisol levels on working memory at effect sizes of: 0.32 (for comparison

load 9); 0.53 (for comparison load 12) and 0.47 (for comparison load 16); this meant that medium-large effect sizes were found for the effects observed at load 9, and large effect sizes were found for the effects observed at loads 12 and 16. Consequently, based on this previous research, the large effect size of 0.78 was considered appropriate for this current study.

All participants recruited into the current study were aged between 18 and 25 years (i.e., they were young to control for the additional effects of age on cortisol and memory performance). The mean ages for participants in the stress vs. control vs. blocker groups were 20.20 (SD = 1.24) vs. 20.45 (SD = 1.47) vs. 20.95 (SD = 1.70) years respectively. There were no significant between-group differences ($F(2,59) = 1.329$; NS) or within-group differences ($F(2,57) = 0.486$; NS) in age. There were also no significant between-group differences ($F(2,59) = 0.468$; NS) or within-group differences ($F(2,57) = 0.521$; NS) in measures of BMI.

Participants recruited into the study were obtained from a convenience sample of students studying at the University of Bristol. Consequently, as this meant that participants had experienced similar levels and periods of education, the potential for task performance to be confounded by intelligence was minimised. (See *Recruitment* for details of the recruitment methods and procedures used.) All participants were paid an honorarium of £20 upon completion of both testing sessions only (i.e., no payments were given for part-completion).

All participants fulfilled the requirements of the exclusion and inclusion criteria. The exclusion criteria stated that all participants should not be taking any current medication and that they had to be either non-smokers or

ex-smokers for at least six months. This was because nicotine-enhanced effects on memory have been reported. For example, Rusted, Graupner, Tennant & Warburton (1998) administered nicotine to minimally deprived smokers and identified nicotine-induced improvements during a semantic recall task. The inclusion criteria stated that all participants had to be healthy, both physically and mentally. Therefore, as part of the induction process, all participants were screened for anxiety and depression. Depression levels were measured using the Beck Depression Inventory (BDI 21; Beck, Ward & Mendelson et al., 1961) and anxiety levels were measured using the General Health Questionnaire (GHQ-30; Goldberg, 1972; Goldberg, 1978; Goldberg & Williams, 1988).

A series of one-way ANOVA's were carried out to see if there were any between-group and within-group differences in these two sets of scores. These showed that the mean BDI scores differed significantly between the three groups ($F(2,59) = 4.070$; $p<0.05$) but not within-groups ($F(2,57) = 2.577$; NS; i.e., participants within each group had similar BDI scores). Post-hoc analysis using Tukey's test for equal variances showed that the significant between-group differences in BDI scores were between the high cortisol and low cortisol groups only ($p<0.02$). The results also showed that the mean GHQ scores differed significantly between-groups ($F(2,59) = 6.192$; $p<0.01$) and within-groups ($F(2,57) = 3.893$; $p<0.05$; i.e., participants within each group had significantly different GHQ scores as well as between-groups). Post-hoc analysis using Dunnett's T3 for unequal variances showed that the significant between-group differences in GHQ scores were between the low cortisol group and both the high ($p<0.01$) and control groups ($p<0.02$). It was

therefore decided that BDI and GHQ scores would be treated as potential covariates during the analyses of the results. (See page 155 for BDI and GHQ group means.)

As part of the inclusion criteria, all participants were also asked if there was any history of serious family illness and whether they suffered from: chronic inflammatory disease; psychiatric disorders; obesity; coronary heart disease; sleep disorders; diabetes (or any other 'abnormal' glucose condition); or any other serious medical condition. Participants who answered positively to any of these questions were not recruited into the study; no participants gave any positive response to this question.

3.4.3. *Materials/Apparatus*

The following quantities are per participant (N = 60).

- Information Sheet, giving details of study (see Appendix V)
- Personal Record Sheet (see Appendix VI)
- Consent Form (see Appendix III)
- Beck Depression Inventory (BDI-21 – see Appendix VII)
- General Health Questionnaire (GHQ-30 – see Appendix VIII)
- Instructions for administering tablets and procedure for each day of testing (see Appendix IX)
- Participant Record sheets (see Appendix X)
- Checklist of items containing caffeine (see Appendix I).

The following quantities are in total.

- Medication (i.e., hydrocortisone [240 x 10 mg tablets], calcium carbonate [30 tablets] and metyrapone [720 tablets]).

- Watch, with second hand, to record timed tasks (i.e., FAS task, Spot the Word Task and Category Naming task).
- IBM Compatible Computer, to present: the Letters Item Recognition task and the Doors and Names recognition tasks.
- Glucose testing kit, comprising Softclix Pro and Lancets (Roche), Accutrend GC System (Boehringer Mannheim), BM Accutest test strips and instructions for use (see Appendix XI).
- Plasters and wipes.
- 120 Salivettes (Sarstedt Ltd, Leicester, UK).

3.4.4. Salivary Cortisol Levels

Each participant was asked to produce one saliva sample upon arrival for each testing session. These samples were collected using salivettes and were used to determine cortisol levels. After collection, all samples were frozen and stored until analysis using the salivary cortisol assay procedure described in Chapter 2.

3.4.5. Glucose Levels

Chapter 1 described how one explanation for the discrepancy in results obtained by Beckwith et al. (1986) compared to those found by Kirschbaum et al. (1996) was the administration of glucose as a placebo. Beckwith et al. used glucose as a placebo and glucose can enhance cognitive performance (Benton et al., 1994; Parker & Benton, 1995; Korol et al., 1995; Parsons & Gold, 1992). Consequently, as the type of food group consumed prior to testing (including quantity) was not controlled for in this current study, participants

were asked to produce a finger-prick sample of blood at the end of each testing session to determine blood glucose levels. The reason for this was to see if there was any relationship between glucose levels and cognitive performance.

All blood samples for the study were obtained using a Softclix Pro and Lancet (Roche). The samples were then analysed using an Accutrend GC system (Boehringer Mannheim) and BM Accutest test strips. This is a very safe and simple system to use, and can produce an accurate glucose reading within twelve seconds. (Refer to Appendix XI for details of the operating instructions.) However, even though the procedure used was described as 'pain-free' in their literature, all glucose testing was carried out at the end of each testing session to avoid any stress-induced increase in cortisol levels.

3.4.6. Other measures

As described previously, all participants were asked to provide details of their:

- Age.
- Height and weight, to calculate BMI.
- Approximate caffeine intake from 24 hours prior to testing.
- List of food items consumed during the day prior to testing.

These data were then available to use as covariates, if appropriate.

3.4.7. Screening Tools

- *Beck Depression Inventory, Version 21 (Beck et al., 1961)*

All participants were screened for depression levels using the BDI 21. This is a 21 item self-rating scale, which defines the cognitive symptoms of depression (see Appendix VII). It also provides a valid measure of symptom

severity in normal, non-clinically depressed students (Bumberry, Oliver & McClure, 1978).

Each copy of the BDI 21 comes with full instructions for completion. These instruct the respondent to select from one of four responses to indicate how s/he has been feeling during 'the past week, including today'. The range of scores for each item runs from 0 (low) to 3 (high), with total scores ranging from 0 to 63 (although this may be higher if multiple responses are given). The scoring guide (based on normative data) is: 0-9 = normal; 10-15 = mild levels of depression; 16-19 = mild/moderate levels of depression; 20-29 = moderate/severe levels of depression; and > 29 = severe levels of depression. The cut-off score for volunteers recruited into this study was > 11 (see Gallagher, Breckenridge, Steinmetz & Thompson, 1983).

The figures for reliability and validity for the BDI 21 are good. A reliability correlation of 0.75 was reported between the BDI and Hamilton Rating Scale (Schwab, Bialow, Brown & Holzer, 1967). The BDI also has high internal consistency and split-half reliability (r=0.86; Beck et al., 1961). For example, Gallagher, Nies & Thompson (1982) identified coefficient alphas of internal consistency of 0.73-0.91. When correlated with other depression inventories, these scores ranged from 0.81 to 0.66. In terms of validity, the BDI correlates well with psychiatrists' assessments and other depression scales. For example, validity correlations between 0.58 and 0.82 were reported between the BDI and the Hamilton Rating Scale (e.g., Miller et al., 1985).

- *General Health Questionnaire, Version 30 (Goldberg, 1972; 1978; Goldberg & Williams, 1988).*

All participants were screened for anxiety levels using the GHQ 30. This was initially designed as a 60-item screening questionnaire for psychiatric disturbance of recent onset (see Appendix VII) and focuses on general symptoms of psychiatric morbidity, in particular depression and anxiety. It also comes with full instructions for completion and was scored using the method recommended by Goldberg, whereby scores of either one or two are assigned to one of four response categories provided for each item, i.e., 1-1-2-2. The cut off score for volunteers recruited into this current study was > 60.

The GHQ is also one of the most extensively tested scales for test-retest reliability (Goldberg, 1978) and validity, and the results produced are good. For example, test-retest correlations range from r=0.51 to 0.90 (Goldberg & Williams, 1988), and internal consistency has been reported to range from 0.77 to –0.93 (Cronbach's alpha). In terms of validity, an analysis of items has confirmed its content validity, and a principle component analysis has shown that there is a large 'general' factor. The factors also tend to cluster together (Murphy et al., 1987). In addition, gender, age and education level shows no significant effect on the validity of the GHQ (Goldberg et al., 1997).

3.4.8. Medication

Full details of the types and doses of medication used for the high and low cortisol conditions are described in Chapter 2 (i.e., hydrocortisone was used to increase cortisol levels and metyrapone was used to reduce cortisol levels). Calcium carbonate was administered in the control condition as this has no

effect on cortisol levels. The doses of medication, times of administration and aims of doses used are summarised in Table IX. Whilst acknowledging the concerns over participant's compliance, all medication was prepared and pre-packed by the hospital pharmacy in the Bristol Royal Infirmary and supplied for self-administration in tablet form.

Table IX : Dosages and Medication used

Medication	Time of Day of administration	Total Dosage (no of tabs)	Reason for dosage
Hydrocortisone (high cortisol condition), to increase cortisol levels to stress levels in the morning and morning levels in the afternoon.	07.00 hrs 08.00 hrs 14.00 hrs 15.00 hrs 16.00 hrs	20 mg (2) 10 mg (1) 5 mg (1/2) 2.5 mg (1/4) 2.5 mg (1/4)	To investigate the effects of moderate-stress levels of cortisol (very high) on memory performance. To investigate the effects of 'normal morning' levels of cortisol (high) on memory performance.
Calcium carbonate (control condition), which does not alter cortisol levels	07.00 hrs 08.00 hrs 14.00 hrs 15.00 hrs 16.00 hrs	1 tablet 1 tablet 1 tablet 1 tablet 1 tablet	To investigate the effects morning levels of cortisol (high) on memory performance. To investigate the effects of afternoon levels of cortisol (low) on memory performance.
Metyrapone (low cortisol condition), to reduce cortisol levels to a minimum and identify where an optimum level of cortisol is necessary for memory performance.	07.00 hrs 14.00 hrs	1500 mg (6) 1500 mg (6)	To investigate the effects of 'minimum' levels of cortisol (very low) on memory performance. To investigate the effects of 'minimum' levels of cortisol (very low) on memory performance.

To prevent participants from interpreting how they might feel (e.g., a participant who knew he had been assigned to the stress condition might expect to feel stressed and, therefore, report feeling stressed) participants were not made aware of the condition they had been allocated to until the debriefing session at the end of the second testing session. For safety reasons, however, the researcher did know which condition each participant had been allocated;

this was in case of any emergency (e.g., adverse reactions to medication). The researcher also gave out a 24-hour contact number to each participant in case of emergency.

3.4.9. Memory Tests

The effects of cortisol on memory performance were tested using two different, but counterbalanced, batteries of memory tests. Each battery comprised two versions of ten different memory tasks, which were each designed to take no longer than 45 minutes to complete. The tasks used, order and method of delivery are shown in Table X. The tasks were always presented in the same order; only the versions of tasks used changed.

Table X : Order of Individual Memory Tasks and Method of Delivery

Task Order	Name of Task	Aspect of memory being tested	Present-ation Method
1	Forward and Backward Digit Span (part of Weschler Adult Intelligence Scale [WAIS] test)	Working Memory	Verbal
2	Item Recognition Task (based on Sternberg, 1966) and replication of task used by Lupien et al., 1999)	Working Memory	Via PC
3	Hopkins Verbal Learning Test (Brandt, 1991)	Episodic Memory	Verbal
4	The Names Recognition Task (Baddeley et al, 1994)	Episodic Memory	Via PC, response verbal
5	The Doors Recognition Task (Baddeley et al., 1994)	Episodic Memory	Via PC, response verbal
6	The Speed of Comprehension Task – Versions A and B (part of SCOLP)	Semantic Memory	Paper and Pen
7	Controlled Oral Word Association (COWA; Benton & Hamsher, 1976, 1989; Spreen & Strauss, 1991)	Indirect measure of working memory (Estes, 1994).	Verbal
8	Letter Naming Task (using letters F, A and S)	Working Memory	Verbal
9	The Spot the Word Task – Versions A and B (part of SCOLP)	Semantic Memory	Paper and Pen
10	Category Naming Task (using categories for animals, fruit and parts of the body)	Working Memory	Verbal

- *Allocation of tasks to each battery*

One version of each of the two versions of memory tasks (with the exception of the item-recognition task) was randomly allocated to each one of two batteries to control for practice effects. (The order of presentation of items in the item-recognition task was automatically randomised by the computer software program.) Allocation of the tasks into batteries was carried out by pseudo-randomisation using five possible combinations of tasks. This is because a total of nine tasks would, otherwise, provide too many possible combinations. The five combinations were randomly selected by withdrawing labels, with each task version written on it, from a bag. The five combinations of tasks were then labelled 1-5, and with both morning and afternoon (i.e., the same labels were used to allocate participants to the two within-groups conditions). Details of the combinations of tasks used and how these were labelled is shown in Table XI.

All ten labels (i.e., 1 – morning, 1 – afternoon, 2- morning, etc.) were then placed in a bag. As each participant was recruited into the study and allocated to each between-group condition, a label was also drawn from a second bag to identify (1), whether the first testing session was a morning or an afternoon session and (2), which battery of memory tasks would be used. Only one label was withdrawn per participant. A label was also withdrawn to identify the first testing session only; the second testing session was made up from those tasks that had not been used in the first session. In addition, with a total of twenty participants in each condition, to avoid duplication, once a label had been withdrawn from the bag it was not returned until after the tenth participant. This meant that two participants in every condition underwent the

same battery of tasks. In addition, to control for time of day order effects, half of the participants in each condition completed the first testing session in the morning and half in the afternoon.

Table XI : Combinations of tasks for each battery

Type of task	Version	Version	Version	Version	Version
Forward Digits (Version 1 or 2)	1	1	1	2	2
Backward Digits (Version 1 or 2)	1	2	1	1	2
Hopkins Verbal Learning (Version 1 or 2)	1	2	2	1	2
Names Task (Version D or G)	D	G	G	D	G
Doors Task (Version 5 or 10)	5	10	5	10	5
Category Naming (Animals/Fruit)	ANIMALS	FRUIT	ANIMALS	FRUIT	ANIMALS
Letter Naming (F or A)	F	A	F	F	A
SCOLP (Version A or B)	A	B	B	A	B
Battery Label[2]	1	2	3	4	5

- ***Working Memory Tasks***

Working memory performance was measured using four different tasks. These comprised: (1) Forward and Backward Digit Span (part of the WAIS test); (2) an item-recognition task (Sternberg, 1966); (3) the FAS test; and (4) a category naming task. The latter two tasks form part of the Controlled Oral Word Association (COWA) test (Benton & Hamsher, 1976; 1989; Spreen & Strauss, 1991).

[2] Each battery was also labelled either MORNING or AFTERNOON

- *Forward & Backward Digit Span*

The distinction between processing speed and short-term capacity reflects the basic dimensions of attention. However, although speed and quantity are related, these two dimensions can be measured separately. Very short-term memory is normally measured using span tests. Under these conditions, participants are subjected to increasingly larger (or smaller) amounts of information, which they have to show they can recall (e.g., by repeating the information). The format of digit span tests used in this current study is the one most commonly used for measuring the span of immediate verbal recall.

The digit span tasks comprise two different tests: the digits forward, and the digits backward. Each of these tasks involve different mental activities and are affected differently by brain damage (see Banken, 1985). Forward digit span is assumed to principally measure the phonological loop, whilst backward span comprises more of an executive component. However, a disparity between scores of >3 is generally only seen in brain damaged individuals rather than 'healthy' individuals.

The digit span tasks used in the current study comprise seven pairs of random number sequences. The investigator read out each sequence at a rate of one digit per second. Consequently, both tasks also involved auditory attention and rely on short-term retention capacity. Two different, but comparative, versions of each task were used in each battery of memory tasks (see Table XI). However, the order in which the tasks were presented was always the same: digits forward – digits backward - digits backward – digits forward. (Refer to Appendix X for examples of randomised digit lists used.) Different sequences of digits were presented on each occasion.

Although there are mixed results, the normal span range for digits forward is considered to be 6 ± 1 (Miller, 1956) with a span of 4 digits considered borderline. Anxiety tends to reduce the number of digits recalled, although this effect may be difficult to identify in the individual case (e.g., Mueller, 1979). Practice effects are statistically significant ($p=0.45$) but negligible, with test-retest reliability coefficients ranging from 0.66 to 0.89, depending on interval length and participant's age (e.g., Youngjohn, Larrabee & Crook, 1992). The digit span task does not tend to be affected by age until beyond ages 65 or 70 (Craik, 1990).

The scores considered to be within the normal range for digits backward are 4 or 5, with 3 considered borderline or defective (e.g., Botwinick & Storandt, 1974). The normal raw score difference between digits forward and digits backward can run from as low as 0.59 to as high as 2 (Strub & Black, 1981).

The digits forward task has been described as being more a task of the efficiency of attention than memory (e.g., Spitz, 1973). However, the additional mental activity required during the digits backward task allows it to be considered much more a task of working memory (e.g., Banken, 1985).

- *Instructions for administering and scoring the digits forward and digits backwards tasks*

The instructions for administering the digits forward and digits backward tasks are detailed in the WMS-III (UK; Wechsler, 1998). In brief, at the start of the task, each participant is told that s/he will be about to hear a series of number sequences, which will be read out by the investigator at a rate of one number

per second, starting with 3 numbers. Two trials per number are read out on each occasion (e.g., 5-8-9, 7-2-4, 9-3-5-2, 6-3-7-5, etc.). The participant is also told that s/he will not receive any feedback or repetition of sequences, and that if s/he cannot remember any numbers to simply say so.

In the digits forward task, after hearing each trial, the participant is told to repeat each sequence back to the investigator (if possible) in exactly the same order as it is heard. Conversely, in the digits backward task, the participant is told to repeat each sequence (if possible) in the reverse order to that which is heard (an example is always given to the participant to make sure they understand this, e.g., if the participant hears 6-9-8, s/he would need to repeat back the sequence 8-9-6). In the current study, each participant was given one practice trial consisting of one two-numbered sequence, for each task. This was to ensure that the correct instructions had been received and understood.

Each task consists of fourteen individual trials, with sequences ranging from three – eight numbers. However, as soon as the participant incorrectly recalls three sequences in succession, the investigator stops the current trial and moves onto the next one. The participant's score is calculated as the total number of correct trials recalled (out of 14) in the correct order (i.e., a total score of 56 may be obtained if the participant recalls everything correctly for all four digit span tasks).

- *Item Recognition Task*

Working memory impairments have been identified using a variety of tasks in which a temporal gap is introduced between a stimulus and a response, thus creating the need to maintain the stimulus in temporary memory storage (Lupien et al., 1999). A similar task was used in this current study, which was an item-recognition task based upon the work carried out by Sternberg (1966). The task used was also a shorter version of the task used by Lupien et al. and, consequently, is described elsewhere (see Lupien et al., 1999).

Sternberg (1966) reported two versions of the item-recognition task. These comprised the varied set procedure and the fixed set procedure. The varied set procedure is where participants are presented with a new target set on each trial. The fixed set procedure is where the same target set is presented for a number of trials; Lupien et al. (1999) used the fixed set procedure in their study. There has, however, been criticism of this procedure. In a previous study, Sternberg (1975) found that a fixed set was still retained in memory two weeks after initial presentation. This implies that the task may be tapping into long-term memory. However, it has also been shown that the completion of this task taps onto the limited-capacity, controlled processing of the central executive processor of working memory in humans (e.g., Kahneman, 1973; Shiffrin & Schneider, 1977). In addition, the cognitive variables used in the task (e.g., variation of processing load) also significantly activate the prefrontal cortex. For a review, see Dolan & Fletcher, (1997) and Smith et al., (1998).

As the study by Lupien et al. is currently the only study reporting the effects of acute changes in cortisol levels on working memory, the same

reaction time task was used to see if these results could be replicated. However, in order to fit within the 45-minute battery of memory tasks, the version of task used in this current study was three times shorter (i.e., it took 10 minutes to complete as opposed to 30). The task used comprised five different comparison loads (i.e., 1, 2, 4, 8 and 16), made up of 108 trials. The one used by Lupien et al. comprised eight different comparison loads (i.e., 2, 3, 4, 6, 8, 9, 12 and 16), made up of 300 trials. Consequently, the task used comprised a series of 12 discrete trials and as each trial 'generally gives rise to reaction times in the order of 500 to 900 msec' (Shiffrin & Schneider, 1977), an entire condition never lasted more than 30 seconds. This is considered to be within 'the time limited capacity of working memory' (Baddeley, 1986; Lupien et al., 1999).

- *Instructions for administering and scoring the item-recognition task*

Full details relating to the task and instructions for administration are described in Lupien et al. (1999). Further details can also be found in Sternberg (1966). In this current study, each participant received instructions via a Microsoft Powerpoint presentation displayed on a computer screen. (Refer to Appendix XII for full details.) To check that these instructions had been understood correctly, each participant was also given a practice session. This comprised three random conditions.

Each condition consisted of the presentation of either 1, 2 or 4 uppercase 'target' letters, which were displayed for a period of six seconds and which the participant was asked to remember (i.e., intentional encoding was used). This was then followed by a 750 msec fixation point (i.e., an *),

followed by the presentation of 1, 2 or 4 search uppercase letter/s. The participant was instructed to respond to the search letter/s by either pressing the yellow key if one of the search letters contained a target letter, i.e., target-present, or to press the red key if there was no target letter present, i.e., target-absent. In a target-present condition, only one target letter would appear. In addition, depending on the number of target letters shown, this one target letter could be different. For example, in a target set comprising the four letters – D S M R, the target letter could either be a D, an S, an M, or an R. Each condition comprised six target-present and six target-absent trials. The order of presentation was randomised as part of the computer software design and the search letters remained on the screen until the participant made a response. Once a response had been made, a second set of search letters then appeared to which the participant had to make their next response. This continued for a total of twelve trials and for nine different conditions, i.e., each participant had to complete a total of 108 trials. The stimuli used for all nine conditions comprised the uppercase letters A, C, D, E, M, R, S, U and Z; these letters sufficiently different from each other not to cause added confusion, i.e., unlike M and N, or P and R.

The processing capacity load was manipulated by varying the number of target letters a participant had to hold in memory and/or by varying the numbers of search letters s/he had to compare them with. For example, with a target set of four target letters and a search set of four search letters, the processing load would be sixteen. The processing load size and number of combination sets are shown Table XII. The order of presentation of these was also randomised as part of the computer software design. The computer

software also recorded the actual responses made and the time in seconds taken to make these.

Table XII : Processing loads and combinations used in item-recognition task

Processing Load Size	Combination sets
1	1 x 1
2	1 x 2 2 x 1
4	4 x 1 1 x 4 2 x 2
8	2 x 4 4 x 2
16	4 x 4

- *Letter Naming Task (FAS test)*

Verbal fluency is typically measured by the quantity of words produced within a restricted category (e.g., from a given letter and within a restricted period of time). In healthy adults, verbal fluency tends to maintain well into the 70 year age range (Benton & Sivan, 1984). Individuals with frontal lobe damage, however, are often unable to develop these word-seeking strategies and, consequently, their verbal fluency becomes impaired (Janowsky, Shimamura, Kritchevsky & Squire, 1989). Verbal fluency also indirectly involves working memory, as the individual also needs to keep track of the words which have already been said (Estes, 1994).

Verbal fluency was measured in this current study using the FAS test. This is a word generation task in which participants are required to produce words according to the letters F, A or S. (These letters are used because of the frequency of English words beginning with these letters.) The FAS version

also forms part of the Neurosensory Center Comprehensive Examination for Aphasia and has been shown to be a sensitive indicator of brain dysfunction (e.g., Miceli, Caltagirone, Gainotti, Masullo & Silveri, 1981).

- *Instructions for administering and scoring the Letter Naming task.*

In this current study, each participant was asked to say out loud as many words beginning with a given letter (i.e., F, A or S) in sixty seconds. He was also told that he could say any word, except for people's names, place names or the same word with a different ending (e.g., runner, running, runs). The participant's score was the total number of words recalled. Repeated words were not included in this score and a note of the number of repeated words was made.

- *Category Naming Task (Animals and Fruit)*

This task is very similar to the FAS test, but in this task the participant is given a category to name words from as opposed to a letter (e.g., animals or fruit). In addition, verbal fluency tasks calling for items in a category provide the structure lacking in those asking for words by an initial letter.

The categories used for this current study were FRUIT and ANIMALS (any animals). The sixty second animal naming task is frequently used with dementia patients and is incorporated into the assessment protocol used by the Consortium for the Establishment of a Registry for Alzheimer's Disease (CERAD; Morris et al., 1989).

- *Instructions for administering and scoring the Category Naming task*

The same procedure for administering the sixty second Letter Naming task was used, except that the participant was given a category to name words from as opposed to a letter. The categories used in this study were either ANIMALS or FRUIT. The score produced was the sum of all acceptable and non-repeated words produced by the participant in sixty seconds.

- ***Episodic Memory Tasks***

The effect of different levels of cortisol on the episodic component of declarative memory (i.e., the 'learning' as opposed to 'knowing' component) was measured using three different episodic memory tasks. These comprised: (1) the Hopkins Verbal Learning Test (Brandt, 1991); and (2) the Names and (3) the Doors Recognition Tasks (Baddeley et al., 1994).

- *The Hopkins Verbal Learning Test (HVLT; Brandt, 1991)*

The HVLT consists of three free-recall trials of a twelve item, semantically categorised list, followed by one trial of yes/no recognition. The lists used were constructed from three semantic categories (e.g., four-legged animals, precious stones and human dwellings) which were selected from among 56 word categories previously studied by Battig & Montague (1969). They were also composed of words of relatively low frequencies of occurrence in printed test (Francis & Kucera, 1982).

The HVLT comes available in six parallel forms, making it particularly valuable when repeated testing is necessary. In addition, during construction, the six recall lists were very closely matched for mean frequency of

occurrence of the words as responses to the category names in the Battig & Montague normative study (F (5,67) = 0.05, NS).

The HVLT was used in the current study because it is very quick and easy to administer. It also requires no more than ten minutes to complete and does not have a ceiling effect (in recall) in neurologically normal subjects (Brandt, 1991). Two versions of the task were used in this study. These comprised the HVLT Form1 (using words taken from categories of four-legged animals, precious stones and human dwellings) and the HVLT Form 5 (using words taken from categories of occupations/professions, sports and vegetables). The HVLT is an American test and the versions used in this study were selected because it was felt that the words in these lists were more 'culturally meaningful' (they were considered to be more 'English' as opposed to American). (Refer to Appendix X for examples of the word lists used.)

- *Instructions for administering and scoring the HVLT*

During the free-recall trials, each participant was instructed to listen to the word list and to try and remember as many of the words as possible (i.e., intentional encoding was used). The investigator then read the words out at a rate of approximately one word every two seconds. The same procedure was repeated on two more occasions. After the third trial, the participant was then told that he would be about to hear a longer list of words. On this occasion, however, after each word the participant had to verbally say either OLD, if he thought the word he heard was a 'target' word (i.e., a word heard previously during the recall trial), or NEW, if he thought the word was a 'distracter' (i.e., a word not heard previously). During the construction of this

recognition trial, half of the distracters had been drawn from the same semantic categories as the targets (related distracters) and half had been drawn from other categories (unrelated distracters).

Each participant was awarded two scores: a recall score and a recognition score. The recall score was calculated as the total number of words recalled for all three recall trials (i.e., a maximum total score of 36 could be obtained). The recognition score was calculated by adding together the 'true positive' scores (i.e., the number of target words correctly recalled) minus the 'false positive' scores (i.e., the number of distracter words incorrectly recalled as target words).

- *The Names and Doors Test (Baddeley et al., 1994)*

The Names and Doors tests are two tasks of verbal and visual recognition which form part of the Doors and People recall and recognition test (Baddeley et al., 1994) that has previously been used to identify hippocampal dysfunction in amnesic patients. The Names test examines verbal recognition. This is achieved by asking participants to identify names that have been previously shown to them. According to Baddeley et al., using names as stimuli means participants are presented with material that is 'both ecologically meaningful, but where coding in terms of meaning or visual imagery seems much less likely than would be the case for unrelated words'. In contrast, the Doors test examines visual recognition in a similar way to the Names test, except that participants are presented with coloured photographs of doors instead of names. According to Baddeley et al., presenting doors as stimuli, whilst still being meaningful, has the additional advantage of being 'visually rich and yet,

provided the distracters are carefully chosen, allow little help from verbal cues'. Consequently, the Names and the Doors tests have each been designed to examine specific areas of recognition (i.e., verbal versus visual).

There are at least six different, but comparative versions of each task. Only two versions were used in this current study and these were selected because of their highest levels of reliability and validity. The versions used were also recommended personally by Professor Alan Baddeley.

- *Instructions for administering and scoring the Names and Doors Tests*

Participants received instructions on how to complete each task via a Microsoft Powerpoint presentation displayed on a computer screen. (Refer to Appendix XIII for full details.) To make sure that the instructions had been correctly understood, participants were also given a single one practice session. This session comprised the presentation of three target doors, which participants had to identify from amongst three additional distracters. The photographs of the doors presented in the practice session were not used in any of the actual tasks.

In the actual Doors task, participants were presented with pictures of twenty different doors. Each door was displayed on the screen for three seconds, which was controlled by the Powerpoint software. After the last target door had been shown (i.e., number 20), participants were then told that they would next see forty pictures of doors, comprising the twenty 'target' doors they had just seen and twenty 'distracter' doors which they had not seen before. Their task was to state, outloud, whether the door was a target or a distracter. If it was a target door, they were told to say OLD, and if it was a

distracter, they were told to say NEW. In addition, participants were also asked to state how confident they felt with their response. To do this, they were told to say either: DEFINITE (if they felt positive about their response); UNSURE (if they thought they might be right but had a bit of doubt); or GUESS (if they had no idea and they were really only guessing). Details of the response instructions were also displayed at the bottom of the screen as each door was displayed. Instructions for completing the Names and Doors tasks were, basically, the same. However, in the Names task only, participants were also instructed to say each name out aloud as it was presented in the target set. At the time, participants were told that this would help them remember the names.

All of the responses made by participants were recorded manually by the investigator using an appropriate response sheet. (Refer to Appendix X for details of the sheets used.) Reaction times were not recorded as this was not designed as a response latency task; participants were made aware that time taken to complete the task was not important.

The order in which the Names and Doors were displayed to participants was written into the Powerpoint programme. Consequently, the order was the same for each participant. The Doors task was always presented before the Names task; however, the versions of tasks used were randomised. Each test was scored by allocating one mark for each correct response. Consequently, a maximum score of 40 could be obtained for each task.

- ***Semantic Memory Tasks***

The effects of different levels of cortisol on the semantic component of declarative memory (i.e., the 'knowing' as opposed to 'learning' component) was measured using the Speed and Capacity of Language Processing (SCOLP) Test (Baddeley, Emslie & Nimmo-Smith, 1992).

- *Speed and Capacity of Language Processing (Baddeley et al., 1992)*

The SCOLP is a quick, sensitive test, which also incorporates a measure of estimated IQ based on language-knowledge. It comprises two different tasks: the Speed of Comprehension test; and the Spot-the-Word test. The Speed of Comprehension test measures the rate of information processing, whilst the Spot-the-Word test provides a framework for interpreting the results of the first test. Consequently, the SCOLP allows the investigator to differentiate between a participant who has always been slow from one whose performance may have become impaired as a result of some other variable (e.g., cortisol levels).

Both tasks show high reliability and validity. For example, a comparison of the Speed of Comprehension test with the National Adult Reading Test (NART) produced high parallel-form reliability (Nelson & Willison, 1991). A comparison of the results produced by the Spot-the-Word test and NART produced a parallel-form reliability of 0.883. Validity correlations of 0.831 and 0.859 have also been found with NART for Versions A and B respectively

Both tasks are available in four versions: A, B, C and D. Only Versions A and B were used in this study and these were randomised across each battery of memory tests.

- *Instructions for administering and scoring the SCOLP*

Full details of the description of the Speed of Comprehension test and the Spot-the-Word test are described elsewhere (see Baddeley et al., 1992). In addition, full instructions for completion are also provided on the front of each task, together with a set of six practice statements that all participants had to complete.

Each version of the Speed of Comprehension test comprises 100 simple statements about the world. Half of the statements are true (e.g., snakes move around the ground searching for food) and half of them are false (e.g., tractors grow in gardens). Participants are instructed to read through as many of the statements as they can in two minutes, placing a tick next to a true statement and a cross next to a false sentence. The raw score is the total number of sentences completed in the two minute period, less any errors. (It is very rare for normal participants to make more than one or two errors.) The raw scores are then referred to a table of normative values and weighted for age, to obtain a scaled score.

Each version of the Spot-the-Word test comprises a total of sixty pairs of words. Only one word in every pair is a true word (i.e., one that would be found in a dictionary). For example, the word-pair PINNACE-STRUMMAGE. As for the Speed of Comprehension test, full instructions for completion, together with a practice session comprising six word-pairs, are

provided on the front of each task. Basically, this instructs participants to work through all of the word-pairs, putting a tick next to the true word in every pair. They are asked to attempt all the questions and to guess if necessary. They are also informed that the task is not timed.

The SCOLP comes with a scoring template of the correct answers. The participant's raw score is the number of correct answers, which is then compared to a table of normative values and weighted for age, to obtain a scaled score.

- *Interpreting the scores*

If the scaled score on the Speed of Comprehension test is lower than the scaled score for the Spot-the-Word test, reference is made to Table 11 (provided with the test) to identify the likelihood of such a discrepancy for that particular vocabulary level. (A copy of this table, together with instructions on how to interpret the scores, is in Appendix X) With participants of 'normal' IQ levels, the Speed of Comprehension scaled score is normally lower than the Spot-the Word scaled score. Indeed, it is normally only the other way around (i.e., the Spot-the-Word scaled score is lower than the Speed of Comprehension scaled score) for participants with a low vocabulary range (i.e., with an IQ score less than 70).

3.4.10. Procedure

- *Recruitment*

Recruitment into the study was carried out by two methods: by poster and by email. Posters were displayed in various departments within the University of

Bristol, advertising for young males interested in taking part in a study looking at the effects of stress hormones on memory (see Appendix XIV). There was no restriction on the 'type' of student, although it was specified that students from the Department of Experimental Psychology should be no more than first year undergraduates. This was to control for any confounding effects of prior knowledge of memory testing procedures on performance levels. All interested volunteers were asked to respond to the posters by printing their names on an attachment sheet, together with contact details (i.e., email address or telephone number).

An email was also sent out to a database of volunteers who had given their details to the Department of Experimental Psychology during the Freshers Fair in September 1999. (See Appendix XV for details of the email sent out.) This database includes the names of students who expressed an interest in taking part in any studies being carried out by the department. For this study, approximately 250 names were selected based on the criteria that they were male, between 18-25 years and non-smokers.

Out of a total of 55 students who signed their names up to the poster, 14 students were recruited (i.e., three of the students were females and 38 students did not continue to show any interest after receiving further information). Out of a total of 307 students who received emails, only 58 replied back asking for further information. Out of these 58, 46 participants were recruited (i.e., 12 students did not show any interest after receiving the information sheet).

- *Procedure for recruitment*

Recruitment into the study was carried out over a twelve month period from October 1999 to September 2000. The investigation was also conducted with the adequate understanding and written consent of all participants and with full ethical approval from the United Bristol Healthcare NHS Trust.

Participants who took part in the study had to complete four stages in the following order: (1) the initial information stage; (2) the induction stage, and (3 and 4) two testing stages. No testing was carried out during May-July 2000 because of the end of year exams and the potential effects of examination stress on memory performance.

- *The Information Sheet*

All volunteers who expressed an interest in the study were emailed an information sheet (see Appendix XV). This gave further details about the design of the study and what participants would be required to do. All volunteers were asked to read this sheet and then, if still interested and fulfilled the requirements of the inclusion criteria, to reply to the investigator with some convenient dates and times for the first induction meeting.

The information sheet informed all volunteers that, in order to take part in the study, they would:

- **Have to commit to one 15 minute induction session PLUS two 45-minute memory testing sessions.** These were carried out over two separate days (i.e., each participant had to attend a total of three sessions). Participants were also informed that the dates for testing would be arranged on days that were convenient to them, but that they must be able

to attend one morning testing session (at either 09.00 or 10.00 hrs) and one afternoon testing session (at 17.00 hrs). They were also informed that there should be a period of approximately five days between each testing session to allow change in baseline cortisol levels to return to normal.

- **Have to take medication on each testing day (** i.e., tablets only needed to be taken on two separate days). Participants were also informed that the tablets would either be: hydrocortisone (a steroid); calcium carbonate (the placebo); or metyrapone (a cortisol-synthesis inhibitor which temporarily reduces levels of cortisol). They were also informed that the doses given would be safe and would not produce any side-effects. However, as a safety precaution, participants were asked to refrain from driving during the period between taking the tablets and testing. They were also informed, however, that they would be safe to drive after testing had been completed.
- **Have to provide a saliva sample prior to each testing session.** This was obtained using a salivette to measure salivary cortisol levels.
- **Have to provide a finger-prick blood sample at the end of the testing session.** This was a safe and pain-free procedure, and was obtained to measure glucose levels.
- **Have to record their approximate caffeine-intake during the 24 hour period prior to testing.** Participants were given a checklist of items containing caffeine to do this (see Appendix I).
- **Have to recall which food items they had eaten during the day prior to testing.** No restrictions regarding types or quantities of food were given.

The information sheet also stated that participants had to meet the following inclusion/exclusion criteria:

- Not be taking steroids for at least six months prior and during testing.
- Not be suffering from any serious medical condition (e.g., coronary heart disease, diabetes, and obesity).
- Not drink alcohol or take any medication (including recreational drugs) during the 24 hours prior to testing.
- Be a non-smoker, or ex-smoker for at least six months.
- Be a fluent English speaker.

They were also informed that they would be paid an honorarium of £20 upon completion of all three sessions and that no sub-payments would be made for part-completion of the study.

- *The Induction Session*

At the beginning of the induction session, each volunteer was asked to provide details of their: (1) age; (2) height and weight; (3) any history of serious illness; and (4) state whether he considered himself to be a HIGH, MODERATE or LOW caffeine user. They also had to complete the BDI and GHQ questionnaires.

Only those volunteers who achieved scores on the BDI and GHQ below the cut-off score (i.e., < 11 for BDI and < 30 for GHQ) and also fulfilled the requirements of the inclusion criteria were recruited as participants into the study; none of the volunteers in this study were excluded. All participants were then asked to sign a consent form and given an

opportunity to ask any questions. Upon completion of this, each participant was allocated a condition and first testing session (i.e., morning or afternoon) using the procedure described in *Allocation of tasks to each battery.* Dates for testing were then arranged and the medication, together with full instructions on what to do on each testing day, were given out (see Appendix IX for details).

- *The Testing Sessions*

Each participant had to complete one morning testing session (i.e., at either 09.00 hrs or 10.00 hrs) and one afternoon testing session. The order of these sessions was randomised as described in *Allocation of tasks to each battery.*

Morning Testing

For each morning testing session, participants were asked to do the following:

For the Hydrocortisone condition

- Get up in time to take TWO tablets at 07.00 hrs (the offer of an early morning call was made available), along with their 'normal' breakfast. They were also instructed not to eat anything after 07.00 hrs, to allow blood sugar levels to settle two hours prior to memory testing.
- Take ONE tablet at 08.00 hrs.
- Arrive at the Clinical Investigation Unit (CIU) in the BRI for 08.45 hrs, whereupon they would be asked to: produce a saliva sample; report how stressed they felt on a scale from 0 (no stress) to 10 (high stress); report what they had eaten for breakfast and return the completed caffeine check-

list; and complete a battery of memory tests at 09.00 hrs. At the end of the testing session, they would also have to provide a 'finger-prick' sample of blood to measure glucose levels.

For the Control condition

- The procedure for this was exactly the same as for the hydrocortisone condition, except that participants were instructed to take ONE tablet at 07.00 hrs and ONE tablet at 08.00 hrs.

For the Low cortisol condition

- The procedure for this was exactly the same as for the hydrocortisone condition, expect that participants were instructed to take all SIX tablets at 07.00 hrs and to arrive at the CIU for testing at 09.45 hrs. Memory testing was carried out at 10.00 hrs.

Afternoon Testing

The format used was exactly the same as for the morning testing, but with the following exceptions:

For the Hydrocortisone condition

- To eat their normal meals up until 15.00 hrs, but not to eat anything after 15.00 hrs to allow glucose levels to settle two hours prior to testing.
- To take 1/2 tablet at 14.00 hrs.
- To take 1/4 tablet at 15.00 hrs.
- To take 1/4 tablet at 16.00 hrs.
- To be available at the CIU for @ 16.45 hrs.

- To commence memory testing at 17.00 hrs.

For the Control condition

- The procedure for this was exactly the same as for the hydrocortisone condition, expect that participants were instructed to take ONE tablet at 14.00 hrs, ONE tablet at 15.00 hrs and ONE tablet at 16.00 hrs.

For the Low cortisol condition

- The procedure for this was exactly the same as for the Hydrocortisone condition, expect that participants were instructed to take all SIX tablets at 14.00 hrs.

A flow diagram showing the design of the study and a summary of the procedure for each condition is shown in Figure 5.

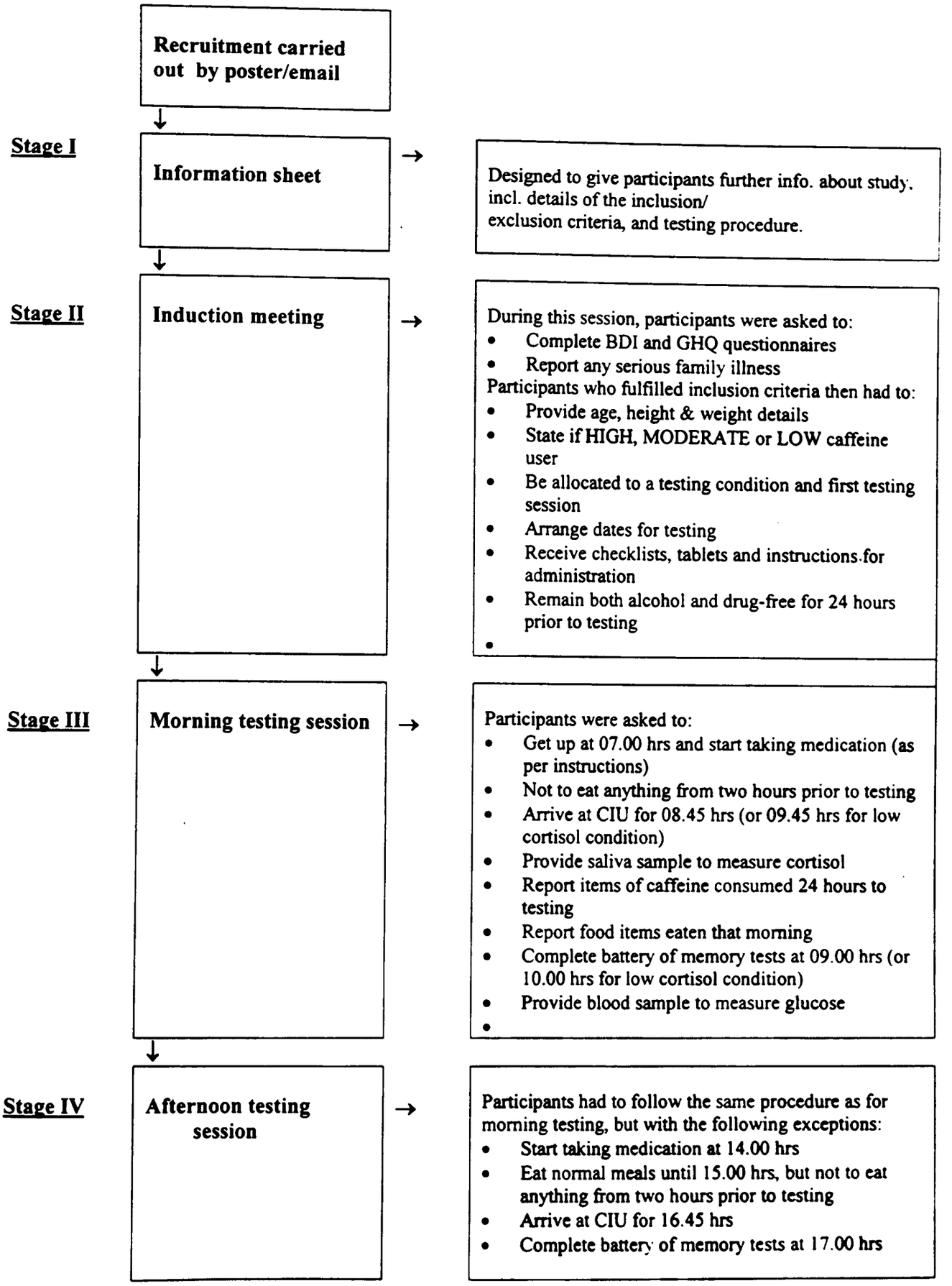
3.4.11. Participant Payments

At the end of the second testing session, all participants were debriefed and paid an honorarium of £20. They were also given an opportunity to ask any questions.

3.4.12. Place of Testing

All testing was carried out in the Clinical Investigation Unit, in the BRI.

Figure 5 : Flow diagram showing a summary of the design of the study.



3.5 Results

The purpose of this study was to examine the effects of different acute changes in cortisol levels on working memory and the episodic and semantic components of declarative memory. Consequently, the most significant data reported in this results section are the actual levels of memory performance produced under each of the three conditions. However, the first part of this results section will focus on the other measures obtained during this study which previous research has shown can modify the effects of cortisol on memory performance. These include the participants' characteristics, levels of caffeine and items of food consumed prior to testing, and glucose levels. The reason for doing this is to show which of these variables modified the effects of cortisol on memory and, consequently, were treated as covariates during the analysis.

3.5.1. Potential Covariates

- *Participant Characteristics*

Table XIII shows the mean age, body mass index (BMI), depression (BDI) and anxiety (GHQ) scores for the high cortisol, control and low cortisol groups.

Table XIII : Mean scores for age, BMI, BDI and GHQ

	High cortisol N = 20		**Control N = 20**		**Low cortisol N = 20**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (yrs)	20.20	1.24	20.45	1.47	20.95	1.70
BMI	21.62	3.10	22.27	3.12	22.49	2.56
BDI	2.95	2.50	2.05	2.16	1.10	1.29
GHQ	50.15	6.20	48.70	4.80	44.85	3.34

As described previously, participants were allocated to each of the conditions randomly using the procedure described in *3.4.2. Participants*. However, although there were no between- or within-group differences in age or BMI, analysis of the results using a series of two-factor ANOVA's (described previously) showed that there were between-group differences between the high cortisol and low cortisol groups in BDI scores. There were also between-group differences between the low cortisol group and both the control and high cortisol groups in GHQ scores, as well as significant within-group differences. It is for this reason, therefore, that BDI and GHQ scores were considered potential covariates in the analyses of these results.

However, for any variable to be treated as a covariate, it must meet each one of three assumptions. These include: (1) being linearly related to the dependent variable (in this case, memory performance); (2) having been measured reliably (in this case, using reliable questionnaires); and (3) producing regression lines for the different groups which are parallel to each other (Dancy & Reidy, 1999). Consequently, as the BDI and GHQ questionnaires which were used to measure depression and anxiety had previously been shown to be reliable, the scores for the BDI and GHQ were analysed to see if the assumptions for (1) and (3) were met. The results produced are as follows:

- *Depression scores and memory performance*

To ascertain whether there was any relationship between depression scores and any of the aspects of memory performance, a series of Pearson's Product Moment correlations were carried out for both times of day (i.e., morning vs.

afternoon). As shown in Table XIV, apart from with the total number of errors made during the item-recognition task in the morning ($r = -0.286$; $p<0.05$), these found no relationships between depression scores and any of the aspects of memory at both times of day. Consequently, as this showed that the BDI scores were not linearly related to memory performance, these were not treated as a covariate in this study.

Table XIV : Showing the non-significant relationships between BDI scores and memory performance.

	Morning		Afternoon	
Memory task	**Pearson's r**	**Sig. Level**	**Pearson's r**	**Sig. Level**
Total digits forward	-0.034	NS	0.106	NS
Total digits backward	0.213	NS	0.170	NS
Item-recognition task (errors)	-0.286	$p<0.05$	-0.020	NS
Item-recognition task (reaction time)	-0.096	NS	-0.090	NS
Letter naming	0.137	NS	-0.051	NS
Hopkins recall	0.116	NS	0.038	NS
Hopkins recognition	0.019	NS	0.087	NS
Names	0.006	NS	-0.018	NS
Doors	0.196	NS	-0.104	NS
Speed of Processing	0.059	NS	0.126	NS
Spot the Word	-0.001	NS	0.013	NS
Category naming	-0.094	NS	0.054	NS

- *Anxiety scores and memory performance*

A series of Pearson's Product Moment correlations were also carried out at both times of day to see if there was any relationship between anxiety scores and memory performance. As shown in Table XV, apart from with the total number of errors made during the item-recognition task in the morning ($r =$

-0.357; $p<0.01$), there were also no significant relationships between anxiety scores and any aspects of memory performance at both times of day. Consequently, as this showed that the GHQ scores were not linearly related to memory performance, these were not treated as a covariate in this study.

Table XV : Showing the non-significant relationships between GHQ scores and memory performance.

	Morning		Afternoon	
Memory task	**Pearson's r**	**Sig. Level**	**Pearson's r**	**Sig. Level**
Total digits forward	-0.075	NS	-0.006	NS
Total digits backward	0.169	NS	0.063	NS
Item-recognition task (errors)	-0.357	P<0.01	0.010	NS
Item-recognition task (reaction time)	-0.051	NS	0.043	NS
Letter naming	0.135	NS	-0.161	NS
Hopkins recall	0.092	NS	0.001	NS
Hopkins recognition	-0.035	NS	0.145	NS
Names	-0.092	NS	-0.102	NS
Doors	0.054	NS	-0.135	NS
Speed of Processing	0.096	NS	0.157	NS
Spot the Word	0.102	NS	0.098	NS
Category naming	-0.155	NS	-0.022	NS

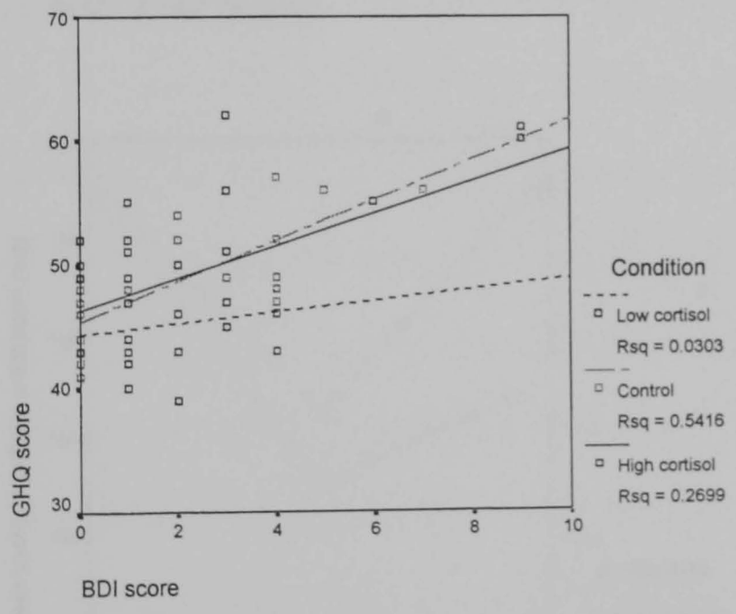
- *Depression scores and anxiety scores*

For both depression and anxiety scores, the only significant relationship to be reported was with the number of errors made during item-recognition performance. Both of these relationships were also negative; this suggests that participants with higher levels of depression and/or anxiety made fewer errors. However, as might be predicted in relation to this, there appeared to be no

effects of depression or anxiety levels on item-recognition reaction time. Also, participants with 'above normal' levels of depression and anxiety were not recruited into the study. Consequently, this suggests that for both of these significant relationships, a Type II error may have occurred.

The relationship between BDI and GHQ scores was also analysed using a Pearson's Product Moment correlation. This showed that BDI scores were positively and strongly related to GHQ scores ($r = 0.606$, $p<0.001$). Thus, as depression levels increase so do anxiety levels. This relationship is shown in Figure 6. This result might have been predicted.

Figure 6 : Relationship between depression and anxiety levels for each group



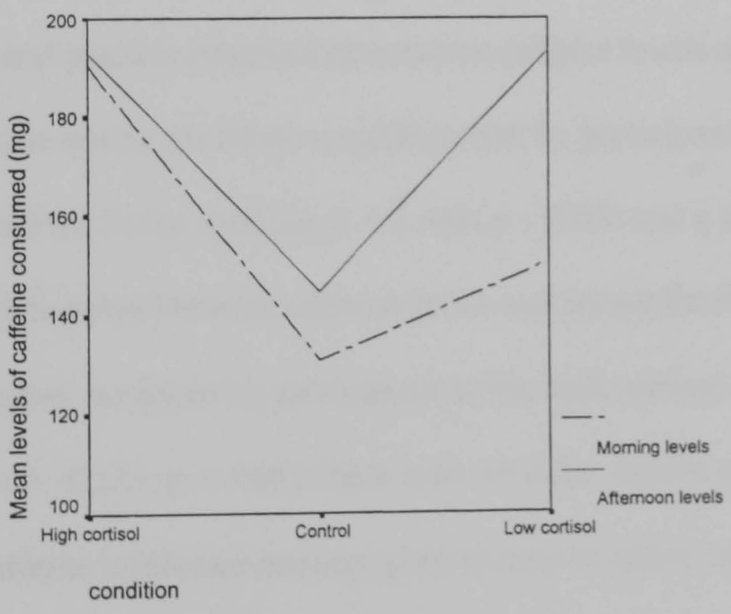
- *Effects of caffeine*

Although the results have been inconsistent, an increase in arousal levels associated with caffeine consumption has been shown to affect memory performance. Consequently, as participants in this study were not told to

refrain from caffeine consumption prior to testing, caffeine levels were considered a potential covariate.

The mean levels of caffeine consumed by participants from 24 hours prior to morning testing were 2.20 (SD = 0.36) vs. 2.21 (SD = 0.34) vs. 1.94 (SD = 0.59) milligrams for the high cortisol vs. control vs. low cortisol groups respectively, in comparison to 2.17 (SD = 0.42) vs. 2.13 (SD = 0.35) vs. 2.13 (SD = 0.53) milligrams in the afternoon. These are shown in Figure 7. As the data were not normally distributed, these were transformed using a logarithmic transformation to achieve normality and, thus, make them appropriate for analysis using a parametric statistical test.

Figure 7 : Showing mean levels of caffeine consumed by each group at both times of day.



Although Figure 7 appears to show group differences in caffeine consumption, the results of a two-factor mixed ANOVA, with time of day (morning vs. evening) and condition (high cortisol vs. control vs. low cortisol) as the two factors, showed no significant main effects of condition ($F(2,43) =$

1.294; NS) or time of day (F = 0.507; df = 1.000; NS). They also showed no significant interaction between condition and time of day (F = 0.377; df = 2.000; NS), which suggests that the level of caffeine consumed was not influenced by the time of day. The results of a Pearson's Product Moment correlation on the transformed data also showed a significantly high and positive relationship between the levels of caffeine consumed by participants in the morning with those consumed in the afternoon ($r = 0.582$; $p < 0.001$). This showed that participants who consumed higher levels of caffeine in the morning also consumed higher levels throughout the day, and vice versa.

A series of Pearson's Product Moment correlations were also carried out between the levels of caffeine consumed and the different aspects of memory performance under each condition. The reason for this was to see if caffeine levels should be treated as a covariate. However, apart from a significant and positive relationship between caffeine levels and total number of errors made during the item-recognition task by participants in the high cortisol condition in the morning ($r = 0.449$; $p < 0.05$) and a significant and negative relationship between caffeine levels and scores for the Hopkins recognition task produced by participants in the high cortisol condition in the afternoon ($r = -0.533$; $p < 0.05$), there were no other significant relationships between caffeine levels and memory performance at either time of day. Consequently, this showed that caffeine levels were not linearly related to memory performance and, therefore, these were not treated as a covariate in this study.

- *Caffeine levels and BMI*

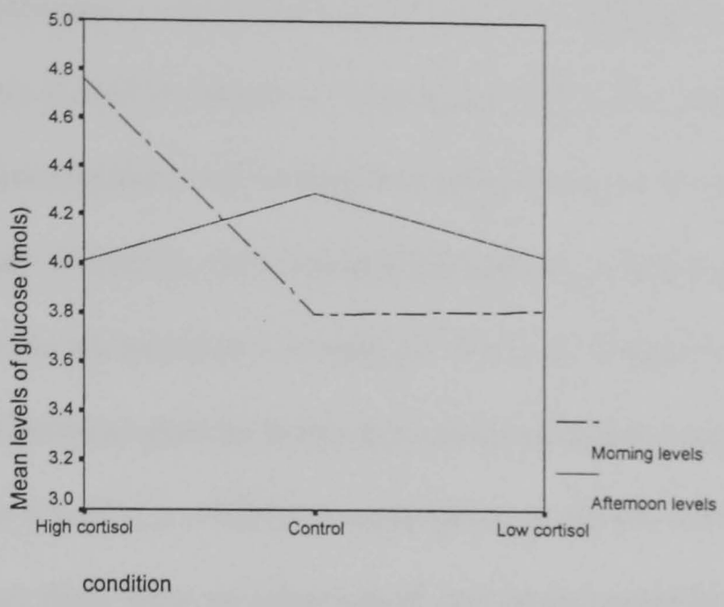
Whilst the results of the Pearson's Product Moment correlations generally showed no significant relationship between caffeine levels and memory performance, a series of Pearson's Product Moment correlations showed a significant negative relationship between both morning caffeine levels and BMI ($r = -0.351$, $p < 0.02$) and between afternoon caffeine levels and BMI ($r = -0.396$, $p < 0.05$). As this showed that high caffeine consumption was associated with a lower BMI, and vice versa, this suggests that caffeine may be a good dieting aid.

- *Effects of glucose*

The mean levels of glucose produced by participants in the morning were 4.66 (SD = 1.10) vs. 3.80 (SD = 1.52) vs. 3.82 (SD = 1.20) mols for the high cortisol vs. control vs. low cortisol groups respectively, in comparison to 4.01 (SD = 0.97) vs. 4.29 (SD = 0.98) vs. 4.04 (SD = 1.11) mols in the afternoon. These are shown in Figure 8. However, although this appears to show group differences in glucose levels, particularly in the morning, the results of a two-factor mixed ANOVA with time of day[3] and condition as the two factors, showed that there were no significant main effects of condition ($F(1,55) = 1.442$; NS) or time of day ($F = 0.005$; $df = 1.000$; NS). There was, however, a significant and positive interaction effect between condition and time of day ($F = 3.488$; $df = 2.000$; $p < 0.05$).

[3] The 'time of day' factors are morning vs. evening, and the factors for 'condition' are high cortisol vs. control vs. low cortisol.

Figure 8 : Showing mean levels of glucose produced prior to testing by each group and at each time of day, together with the interaction effect.



As shown in Figure 8, this suggests that the levels of glucose produced depend on the time of day. More specifically, that participants in the control and low cortisol groups produced lower levels of glucose in the morning compared to the afternoon, whereas the participants in the high cortisol group produced higher levels of glucose in the morning compared to the afternoon. One of the functions of cortisol in the body's response to stress is to increase the levels of glucose that are released into the bloodstream to prepare the body for fight or flight (Stone et al., 2001). These results suggest that a similar effect might have occurred when the cortisol levels were increased using steroids in the morning in the high cortisol condition. They do not, however, explain why the levels of glucose produced following the administration of steroids in the afternoon were lower in the high cortisol group in the afternoon compared to the control group, although this may be an effect of time of day, as the aforementioned results suggest.

A series of Pearson's Product Moment correlations were also carried out between the levels of glucose produced and the different aspects of memory performance under each condition. The reason for this was to see if glucose levels should be treated as a covariate. However, apart from a significant and negative relationship between glucose levels and total number of errors made during the item-recognition task by participants in the control condition in the afternoon ($r = -0.649$; $p < 0.01$) and a significant and positive relationship between glucose levels and scores produced using the Spot the Word task ($r = 0.471$; $p < 0.05$) by participants in the low cortisol condition in the afternoon, there were no other significant relationships between glucose levels and any aspects of memory performance at either time of day. Consequently, as this showed that glucose levels were not linearly related to memory performance, these were not treated as a covariate in this study.

- *Effects of food-type*

Participants were not told which items they should and shouldn't eat during the day prior to testing in this current study. Consequently, because previous research has reported effects of food-group (i.e., high carbohydrates vs. high proteins) on cortisol levels and memory performance, all participants were asked to report which items of food they had eaten prior to testing. The results produced are shown in Table XVI.

A two-factor mixed ANOVA, with time of day and condition as the two factors, was carried out to see if there were any significant differences in the types of food eaten by participants between and within the three conditions. The results of this showed that there were significant main effects

of condition ($F(2,57) = 6.6617$; $p < 0.01$) and significant interaction effects of condition and time of day ($F = 4.724$; $df = 2.000$; $p < 0.05$). There were, however, no significant main effects of time of day ($F = 0.084$; $df = 1.000$; NS).

Table XVI: Showing food-groups eaten by participants in each group during the day prior to testing as percentages

	Condition					
	High cortisol		Control		Low cortisol	
Food-group	**Am**	**Pm**	**Am**	**Pm**	**Am**	**Pm**
High carbohydrate/Low protein	80%	15%	40%	0%	70%	10%
High protein/low carbohydrate	5%	80%	0%	95%	15%	85%
No Food	15%	5%	60%	5%	15%	5%

The results of a post-hoc comparison using Tukeys also showed that there was a significant difference in the types of food-group consumed between the control group and both the high cortisol ($p < 0.01$) and low cortisol groups ($p < 0.05$) in the morning. This was because most of the participants in the control group did not eat any breakfast at all.

A series of Pearson's Product Moment correlations were also carried out to see if there were any relationships between the types of food consumed and memory performance at both times of day under each condition. However, apart from a significant and positive relationship between food-type and number of errors made during the item-recognition task by participants in the control group in the morning ($r = 0.512$; $p < 0.05$) and the significant and negative relationship between food-type and scores on the Hopkins

recognition task produced by participants in the low cortisol group in the afternoon ($r = -0.469$; $p < 0.05$), no other significant relationships between food type and memory performance at either time of day were found. Consequently, as this showed that the types of food consumed prior to testing were not linearly related to memory performance, these were not treated as a covariate in this study.

- *Summary of potential covariates*

In summary, therefore, the results show that, generally, none of the variables previously shown to influence the effects of cortisol on memory showed any relationship with any of the aspects of memory performance in this study. Consequently, there were no covariates used in the analyses of these results.

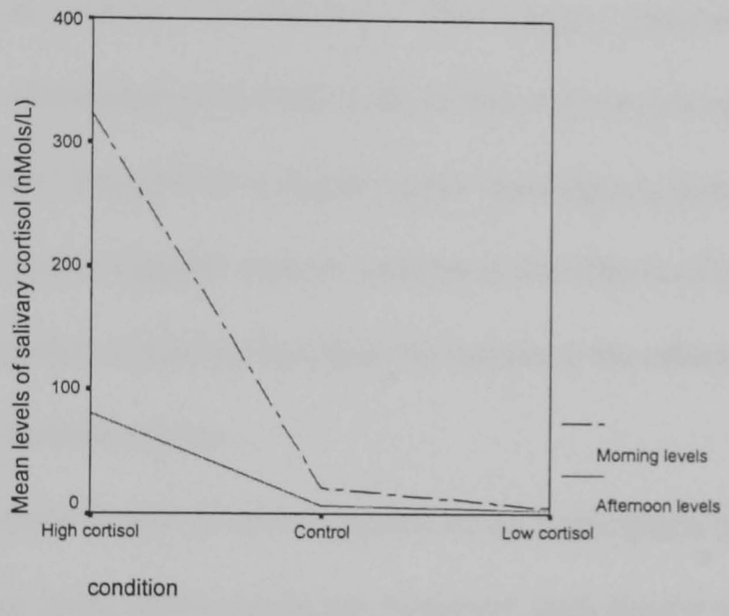
3.5.2. *Salivary cortisol*

Figure 9 shows the mean levels of salivary cortisol produced by each group of participants following the administration of the different types of medication at both times of day. The mean levels produced in the morning were 323.74 (SD = 179.40) vs. 20.92 (SD = 14.31) vs. 4.68 (SD = 3.79) nMols/L for the high cortisol vs. control vs. low groups respectively. In contrast, the mean levels produced in the afternoon were 81.44 (SD = 68.97) vs. 6.6 (SD = 3.03) vs. 2.34 (SD = 1.47) nMols/L.

As the salivary cortisol data were not normally distributed, these were transformed using a logarithmic transformation to achieve normality and, thus, make them appropriate for analysis using a parametric statistical test. A two-factor mixed ANOVA, with time of day and condition as the two factors was

then carried out on the transformed data. This showed highly significant main effects of condition ($F(2,57) = 366.874$; $p<0.001$) and time of day ($F = 78.113$; $df = 1.000$; $p<0.001$), as well as a significant interaction effect between the two ($F = 4.683$; $df = 2.000$; $p<0.05$). Post hoc analysis using Tukeys also identified highly significant differences between all of the groups with each other ($p<0.001$).

Figure 9 : Mean levels of salivary cortisol produced by each group and both times of day



As shown in Figure 9, the results produced were in the predicted direction. More specifically, the levels of salivary cortisol produced by participants in the high cortisol condition were higher than those produced by participants in the control condition, which were higher than those produced by participants in the low cortisol condition. In addition, the levels of salivary cortisol produced by participants in the morning were consistently higher than those produced in the afternoon. This was predicted for the high cortisol and control conditions. Indeed, the levels of cortisol produced by participants in the control group were also similar to those identified by Aardal & Holm

(1995) who reported endogenous cortisol levels between 3.5 – 27.0 nMols/L (i.e., ~ 1.0 – 8.0 ng/ml) at 08.00 hrs and less than 6.0 nMols/L (i.e., ~ 0.1 – 1.0 ng/ml) at 22.00 hrs. The difference between morning and afternoon levels shown by participants in the low cortisol condition, however, was not predicted. This is because it was anticipated that the same dose of metyrapone administered at both times of day would have also reduced cortisol levels to same levels at both times of day. However, the levels of cortisol produced in the morning were actually two times higher than those produced in the afternoon (i.e., 4.68 vs. 2.34 nMols/L). This suggests that the effects of the same doses of metyrapone at both times of day may have been influenced by baseline levels of cortisol (i.e., higher in the morning compared to the afternoon). It also suggests that, in contrast to the effects of hydrocortisone, the administration of metyrapone does not suppress the effects of circadian variation in cortisol release.

The mean levels of cortisol produced by participants in the high cortisol group in the afternoon do not, however, look similar to those produced by participants in the control condition in the morning (i.e., mean = 81.44 vs. 20.92 nMols/L). This was not predicted, as it was intended that the administration of 10 mg hydrocortisone in the afternoon would produce the same levels of cortisol produced normally in the morning (i.e., between 3.5 – 27.0 nMols/L). This suggests, therefore, that as the levels of cortisol were different, a comparison of the memory performance levels between the high cortisol group in the afternoon vs. the control group in the morning would also be different.

A Pearson's Product Moment correlation was carried out to see if there was any relationship between morning salivary cortisol levels and afternoon salivary cortisol levels. The results of this showed a highly significant and positive relationship (i.e., $r = 0.453$; $p<0.001$) between the two, with participants who produced higher levels of cortisol in the morning consistently producing higher levels in the afternoon. A series of Pearson's Product Moment correlations were also carried out to see if there were any relationships between salivary cortisol levels and any aspects of memory performance under each condition. However, apart from significant and positive relationships between salivary cortisol levels and scores using the Hopkins recall task ($r = 0.568$; $p < 0.01$) and Doors task ($r = 0.469$; $p < 0.05$) produced by participants in the Control group in the morning, and the significant and negative relationship between salivary cortisol levels and Speed of Processing task scores produced by participants in the high cortisol condition in the afternoon ($r = -0.445$; $p < 0.05$), no other significant relationships between salivary cortisol levels and memory performance at either time of day were found. Consequently, these results suggest that, generally, there is no relationship between salivary cortisol levels per se. and levels of memory performance following acute changes in cortisol.

- *Individual Differences in cortisol-response*

A closer examination of the range of salivary cortisol levels produced by participants in each condition and at both times of day showed considerate variance in the levels produced. Indeed, as shown in Figure 10, the range of cortisol levels produced by participants within each condition and at both times of day was highly significant. More specifically, a Levene's test for

homogeneity of variance showed highly significant within-group differences in cortisol-response in the morning (i.e., $F(2,57) = 36.418$; $p<0.001$) and in the afternoon ($F(2,57) = 35.687$); $p<0.001$).

It is interesting to note that, even when cortisol levels were manipulated using the same doses of medication, there were still individual differences in cortisol-response. As there is generally large inter-individual variability per se., this suggests that the rate at which the individuals absorbed the hydrocortisone was different and that the sensitivity of the individuals' enzymes to metyrapone was also different. As described in Chapters 1 and 2, previous researchers also identified individual differences in cortisol response following the administration of hydrocortisone (i.e., Bohnen et al., 1990; Kirschbaum et al., 1996; Lupien et al., 1997).

To investigate whether the effects of acute changes in cortisol on memory performance were influenced by whether an individual was a high- or a low-cortisol responder, a series of one-way ANOVA's were carried out on the high-cortisol responders versus low-cortisol responders. Participants who produced salivary cortisol levels above the mean for their group were classified as high-responders, whereas low-responders were classified as those who produced salivary cortisol levels below the mean. The numbers of high- versus low-responders and means for each group are shown in Table XVII.

Figure 10 : Showing the distribution of cortisol levels produced by participants in each condition and at both times of day.

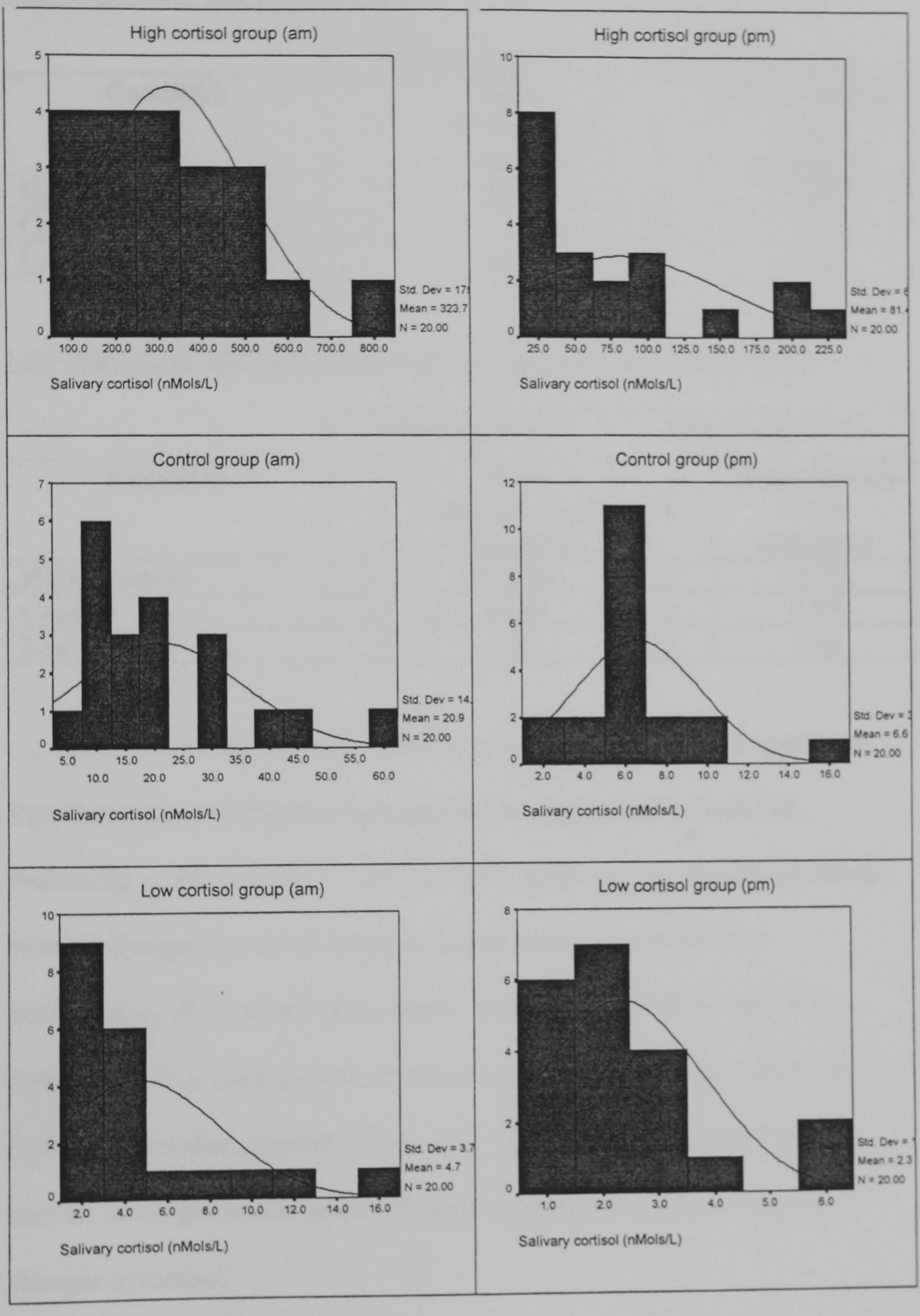


Table XVII : Numbers of high- versus low-responders and mean salivary cortisol levels produced by each group

High-responders who produced higher than mean salivary cortisol levels

Condition	Morning levels		Afternoon levels	
	N	Mean salivary level (nmols/L)	N	Mean salivary levels (nmols/L)
High cortisol	9	483.91	8	150.00
Control	8	34.25	8	9.01
Low cortisol	5	10.24	7	3.80

Low-responders who produced lower than mean salivary cortisol levels

Condition	Morning levels		Afternoon levels	
	N	Mean salivary level (nmols/L)	N	Mean salivary levels (nmols/L)
High cortisol	11	192.68	12	35.73
Control	12	12.03	12	4.99
Low cortisol	15	2.82	13	1.56

The results of the one-way ANOVA's, however, showed that apart from significant group differences between the low-responders in Speed of Processing scores ($F(2,36) = 3.602$; $p<0.05$), there were no significant effects of acute changes in cortisol levels on any of the aspects of memory performance. (Full details of the results produced by SPSS are shown in Appendix XVI.) Consequently, the results of this phase of the analysis also further suggest that, generally, there is no relationship between salivary cortisol levels per se. and levels of memory performance following acute changes in cortisol.

- *Anxiety levels and salivary cortisol*

As described in Chapter 2, Brown et al. (1996) suggested that individual differences in cortisol-response may be related to anxiety levels. Consequently, a series of Pearson's Product Moment correlations were carried out on the transformed data for the total population (i.e., irrespective of condition) to see if there were any relationships between GHQ scores and salivary cortisol levels at both times of day. As shown in Figures 11 and 12, these found highly significant and positive relationships between GHQ scores and salivary cortisol levels in the morning ($r = 0.410$; $p = 0.001$) and in the afternoon ($r = 0.298$; $p < 0.05$). Consequently, although the relationship between salivary cortisol levels and GHQ scores are not the same for each condition, overall these results support Brown et al. by suggesting a positive relationship between anxiety levels and salivary cortisol.

Figure 11 : Showing relationship between anxiety levels and morning salivary cortisol levels

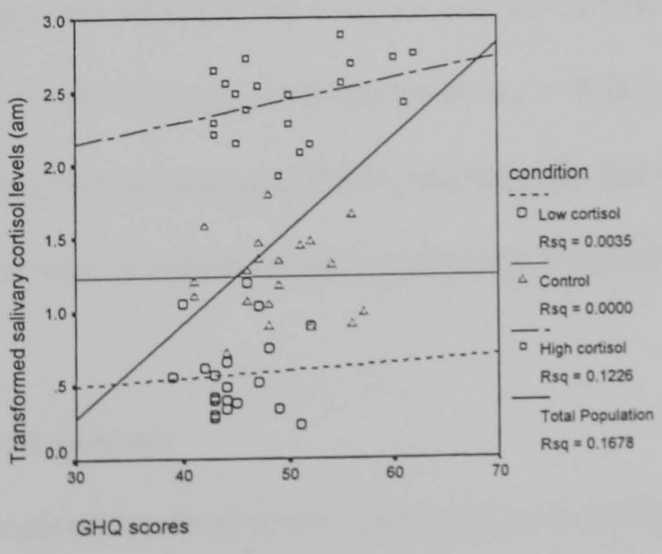
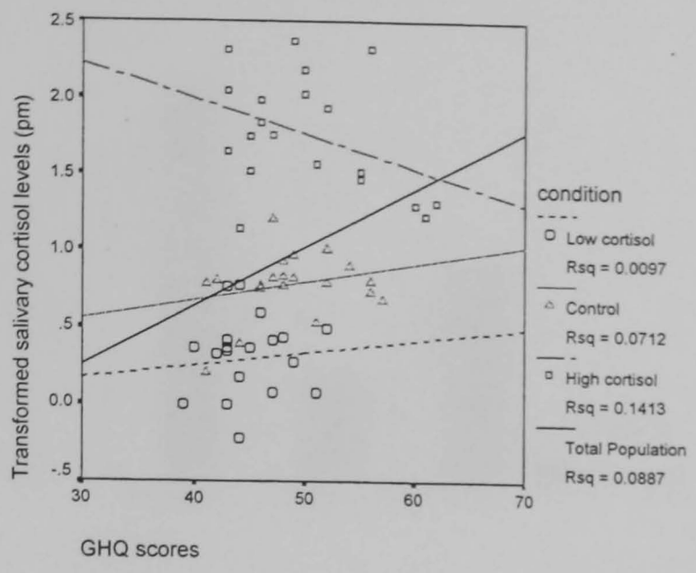


Figure 12 : Showing relationship between anxiety levels and afternoon salivary cortisol levels



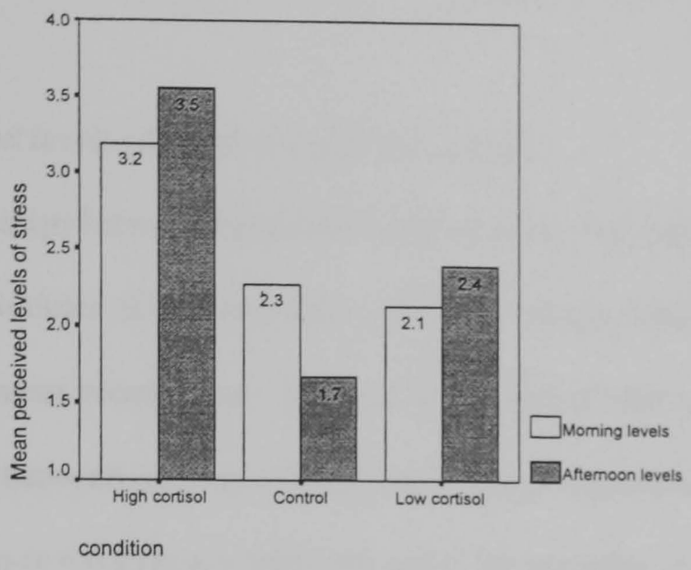
- *Body Mass Index and salivary cortisol*

As mentioned previously, the doses of medication administered for each of the three conditions were the same for all participants irrespective of BMI. Consequently, two Pearson's Product Moment correlations were carried out to see if these same levels of medication produced similar levels of cortisol irrespective of body size. The results showed that there was no significant relationship between BMI and the levels of salivary cortisol produced in the morning ($r = -0.154$; NS) and in the afternoon ($r_p = -0.025$; NS). Although this may be different for extreme BMI's, this suggests that the same dose of medication had similar effects on each participant's cortisol levels.

3.5.3. *Perceived levels of stress*

The mean perceived levels of stress reported by each group at both times of day using a likert rating scale from 0 (indicating no stress) to 10 (indicating high stress) are shown in Figure 13.

Figure 13 : Showing a comparison of perceived stress levels between between groups at both times of day



This shows that there were between-group differences in perceived levels of stress, with the high cortisol group producing the highest perceived levels overall. Indeed, the results of a two-factor ANOVA, with condition and time of day as the two factors, showed that although there were no significant main effects of time of day ($F = 0.001$; $df = 1.000$; NS) or interaction between time of day and condition ($F = 1.679$; $df = 1.000$; NS), there were significant main effects of condition ($F(2,57) = 4.536$; $p < 0.05$). The results of a post-hoc comparison using Tukey's also showed that there were significant differences in perceived levels of stress between the high cortisol and control conditions only ($p<0.05$). Although the lack of significant time of day effects between the high cortisol and control groups were not predicted, these might have been predicted in the blocker condition. The results of a Pearson's Product Moment correlation comparing perceived levels of stress in the morning with those produced in the afternoon also showed that, irrespective of condition, the participants who perceived higher levels of stress in the morning

also perceived higher levels of stress in the afternoon, and vice versa ($r = 0.518$; $p<0.01$). This suggests a consistent behaviour pattern.

- *Perceived levels of stress and salivary cortisol*

The relationships between perceived levels of stress and salivary cortisol levels at both times of day were also examined using a series of Pearson's Product Moment correlations. These showed a significant and positive relationship between salivary cortisol levels and perceived levels of stress in the afternoon ($r = 0.278$; $p < 0.05$) but not in the morning ($r = 0.244$; NS). However, this second relationship was only just non-significant at $p = 0.06$.

As mentioned at the start of this section, the results pertaining to the control issues in this thesis concern the levels of memory performance produced under each of the three conditions. These are now presented in the following section.

3.5.4. Effects of condition and associated cortisol levels on memory performance

A summary of the groups' mean scores and standard deviations for the individual memory tasks completed at both times of day is shown in Tables XVIII and XIX. In the high cortisol condition, it was predicted that memory performance would be impaired in the morning (when cortisol levels were at higher levels) compared to the afternoon (when cortisol levels were at lower morning levels). However, the mean scores for each aspect of memory and at both times of day do not support this.

Table XVIII : Showing mean scores and standard deviations for all tasks completed during the morning testing sessions between groups

Condition →	**High cortisol**		**Control**		**Low cortisol**	
Morning scores ↓	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Total digits forward	23.75	2.67	23.50	3.09	23.80	2.19
Total digits backward	18.00	5.34	18.70	3.92	16.75	3.52
Item recog. – errors	4.35	2.41	3.40	2.35	4.75	2.27
Item recog. – time	79744	11417	80181	9975	85139	13590
Letter naming	15.65	3.65	17.15	5.58	14.75	3.52
Hopkins recall	30.30	3.39	29.00	2.55	28.60	3.89
Hopkins recognition	11.65	0.59	11.50	0.95	11.55	0.83
Names	35.45	3.99	34.25	3.58	35.85	2.98
Doors	35.05	3.58	35.00	3.58	34.00	3.93
Speed of Processing	13.60	2.54	14.20	2.78	12.95	2.54
Spot the Word	12.17	2.13	13.45	2.06	13.10	2.05
Category naming	21.90	7.89	21.10	5.92	20.45	6.35

Table XIX : Showing mean scores and standard deviations for all tasks completed during the afternoon testing sessions between groups

Condition →	**High cortisol**		**Control**		**Low cortisol**	
Afternoon scores ↓	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Total digits forward	24.30	2.23	24.15	3.28	24.00	2.15
Total digits backward	18.00	4.12	19.65	3.86	17.35	3.41
Item recog. - errors	5.40	4.63	4.05	2.28	4.50	2.65
Item recog. - time	79702	13700	80544	14566	86590	13952
Letter naming	15.80	5.42	17.60	5.16	16.60	4.33
Hopkins recall	30.10	3.60	29.30	3.63	28.40	4.19
Hopkins recognition	11.70	0.57	11.60	0.60	11.60	0.68
Names	35.75	3.14	34.85	2.66	35.20	2.97
Doors	34.45	3.07	33.95	4.82	35.05	3.30
Speed of Processing	14.35	2.56	14.95	2.87	13.15	2.13
Spot the Word	12.50	2.28	12.85	1.84	13.15	2.11
Category naming	20.45	6.74	22.45	5.32	21.65	4.27

- *Effects of cortisol on working memory*

A series of two-factor mixed ANOVA's, with time of day (morning vs. evening) and condition (high cortisol vs. control vs. low cortisol) as the two factors were carried out on each of the different types of working memory scores.

- For Total Digits Forward, the results showed no significant main effects of time of day (F = 2.901, df = 1.000; NS) or condition (F(2,57) = 0.035; NS), or any two way interaction effect of time of day and condition (F = 0.248; df = 2.000; NS).
- For Total Digits Backward, the results showed no significant main effects of time of day (F = 1.237, df = 1.000; NS) or condition (F(2,57) = 1.6931 NS), or any two way interaction effect of time of day and condition (F = 0.357; df = 2.000; NS).
- For the number of detection errors made during the item recognition task, the results showed no significant main effects of time of day (F = 1.114; df = 1.000; NS) or condition (F(2,57) = 1.406; NS), or any two way interaction effect of time of day and condition (F =0.075; df = 2.000; NS).
- For the total reaction time taken to complete the item recognition task, the results showed no significant main effects of time of day (F = 0.128; d = 1.000; NS) or condition (F(2,57) = 1.789; NS), or any two way interaction effect of time of day and condition (F = 0.073; df = 2.000; NS).
- For letter naming, the results showed no significant main effects of time of day (F = 1.431; df = 1.000; NS) or condition (F(2,57) = 1.252; NS), or any two way interaction effect of time of day and condition (F = 0.589; df = 2.000; NS).

- For category naming, the results showed no significant main effects of time of day ($F = 0.081$; $df = 1.000$; NS) or condition ($F(2,57) = 0.222$; NS), or any two way interaction effect of time of day and condition ($F = 0.501$; $df = 2.000$; NS).

In summary, therefore, there were no significant main effects of condition or time of day, or any interaction two-way effect between time of day and condition, for any of the working memory tasks.

- *Effects of cortisol on episodic memory*

A series of two-factor mixed ANOVA's, with time of day (morning vs. evening) and condition (high cortisol vs. control vs. low cortisol) as the two factors were carried out on each of the different types of episodic memory scores.

- For Hopkins Recall, the results showed no significant main effects of time of day ($F = 0.006$; $df = 1.000$; NS) or condition ($F(2,57) = 1.314$; NS), or any two way interaction effect of time of day and condition ($F = 0.160$; $df = 2.000$; NS).
- For Hopkins Recognition, the results showed no significant main effects of time of day ($F = 0.333$; $df = 1.000$; NS) or condition ($F(2,57) = 0.281$; NS), or any two way interaction effect of time of day and condition ($F = 0.021$; $df = 2.000$; NS).
- For the Names task, the results showed no significant main effects of time of day ($F = 0.025$, $df = 1.000$; NS) or condition ($F(2,57) = 1.066$; NS), or any two way interaction effect of time of day and condition ($F = 0.516$; $df = 2.000$; NS).

- For the Doors task, the results showed no significant main effects of time of day ($F = 0.092$; $df = 1.000$; NS) or condition ($F(2,57) = 0.938$; NS), or any two way interaction effect of time of day and condition ($F = 0.941$; $df = 2.000$; NS).

In summary, as for working memory performance, there were no significant main effects of condition or time of day, or any two-way interaction effects between condition and time of day, for any of the episodic memory tasks.

- *Effects of cortisol on semantic memory*

A series of two-factor mixed ANOVA's, with time of day (morning vs. evening) and condition (high cortisol vs. control vs. low cortisol) as the two factors were carried out on each of the different types of semantic memory scores.

- For Speed of Processing, the results showed a significant main effect of time of day ($F = 5.472$; $df = 1.000$; $p < 0.05$), with participant's performing better in the afternoon than in the morning ($p < 0.05$). However, there was no significant main effect of condition ($F(2,57) = 2.037$; NS), or any two way interaction effect of time of day and condition ($F = 1.008$; $df = 2.000$; NS).
- For Spot the Word, the results showed no significant main effects of time of day ($F = 0.902$; $df = 1.000$; NS) or condition ($F(2,57) = 0.584$; NS), or any two way effect of time of day and condition ($F = 0.517$; $df = 2.000$; NS).

In summary, whilst the results showed a significant time of day effect on speed of processing performance, there were no other significant effects.

Taken together, therefore, as for working memory and episodic memory, there were no significant main effects of condition or any two-way interaction effects between condition and time of day for any of the semantic memory tasks.

3.5.5. *Effects of inverted U-shaped relationship between corticosteroids and different aspects of memory performance*

As described in Chapter 1, according to the inverted U-shaped relationship between corticosteroids and memory performance, there is an optimum level of cortisol at the peak of the inverted curve which is believed to either facilitate or, at the very least, have a neutral effect on memory performance. However, if this level is either exceeded or not reached, memory performance may be impaired. Consequently, as part of the rationale for this study was to further examine this relationship, a comparison of the results produced by participants in the treated groups (i.e., participants in the high- and low-cortisol conditions, whose levels of cortisol would be classified as being above and below the optimum point of the curve) with those produced by participants in the non-treated group (i.e., participants in the control condition) was carried out. More specificially, a series of two-way mixed ANOVA's, with condition (treated vs. non-treated) and time of day (am vs. pm) as the two factors, were carried out on each of the sets of memory scores. The results produced are shown in Table XX. This shows that, apart from the significant main effect of time of day on speed of processing performance, no other significant main or interaction effects of cortisol on memory performance were found.

In conclusion, therefore, the results of this study did not identify any significant effects on any of the three aspects of memory performance as a function of cortisol levels, time of day (apart from for speed of processing performance), or any two-way interactions between condition and time of day.

Table XX : Showing results of two-way ANOVA's comparing memory performance levels between the treated and non-treated conditions at both times of day.

	Measured effect					
	Main effect of condition		**Main effect of time of day**		**Interaction between condition and time of day**	
Type of task	**F**	**Sig level**	**F**	**Sig level**	**F**	**Sig level**
Working Memory tasks:						
Total digits forward	0.044	NS	3.150	NS	0.227	NS
Total digits backward	2.727	NS	1.630	NS	0.441	NS
Item recog. – errors	0.835	NS	3.150	NS	0.227	NS
Item recog. – time	2.733	NS	0.161	NS	0.066	NS
Letter naming	0.559	NS	0.094	NS	0.010	NS
Category naming	0.440	NS	0.203	NS	0.295	NS
Episodic Memory tasks:						
Hopkins recall	0.046	NS	0.013	NS	0.325	NS
Hopkins recognition	0.244	NS	0.381	NS	0.042	NS
Names	2.160	NS	0.147	NS	0.489	NS
Doors	0.053	NS	0.349	NS	0.834	NS
Semantic Memory tasks:						
Speed of Processing	2.576	NS	5.696	$p<0.05$	0.287	NS
Spot the Word	0.335	NS	1.483	NS	0.897	NS

3.6. Discussion

The overall aim of the current study was to examine the immediate effects of acute changes in cortisol levels (both increased and decreased) on working memory and the episodic and semantic components of declarative memory. The effects were also examined in the morning and in the afternoon. This was to investigate the additional effects of time of day and gain further insight into the inverted U-shaped relationship between cortisol levels and memory performance, which have previously been shown to influence the direction and magnitude of effects produced. Whilst significant differences in salivary cortisol between the three groups and at both times of day were observed, the results failed to find any differences in immediate working memory and immediate declarative memory performance as a function of cortisol levels. More specifically, and in relation to previous research, the results failed to find any evidence to suggest that working memory is more sensitive to acute changes in cortisol levels than declarative memory (Lupien et al., 1999), and that acute changes in cortisol levels have any effects on the episodic and semantic components of declarative memory. However, whilst the results also failed to find any differences in either working memory or declarative memory as a function of time of day (Fehm-Wolfsdorf et al., 1993), they did identify a significant and positive relationship between cortisol levels in the control group and two measures of episodic declarative memory (i.e., Hopkins recall performance and performance on the Doors task) in the morning. Although not consistent across all episodic memory tasks, this suggests that increased endogenous cortisol in the morning facilitates this aspect of memory. The results also identified a significant and negative relationship between cortisol levels in the high cortisol group and one measure of semantic declarative memory (i.e., speed of processing performance) in the afternoon. This suggests that increased cortisol

levels in the afternoon impaired this aspect of memory. The results of the current study also found evidence to support a significant and positive relationship between cortisol levels and perceived levels of stress in the afternoon (e.g., van Eck et al., 1994; and Lupien et al. 1998), as well as between anxiety levels and cortisol-response (Brown et al., 1996). These results are discussed.

3.6.1. Effects of acute changes in cortisol levels on declarative memory

Chapter 1 described how compared to the effects of chronic changes in cortisol levels on declarative memory, the effects of acute changes are less clear. Indeed, whilst numerous studies have investigated the effects of chronic changes in cortisol (e.g., Lupien et al., 1994, 1997; Newcomer et al., 1994; Seeman et al., 1997), far fewer have explored the effects of acute changes (e.g., Kirschbaum et al., 1996; Newcomer et al., 1999; Schmidt et al., 1999). Moreover, whereas the common finding is that chronic elevations in cortisol levels impair declarative memory, the effects produced following acute elevations have been mixed. For example, as described in Chapter 1, Kirschbaum et al. (1996) identified detrimental effects on declarative memory following the administration of 10 mg hydrocortisone. No effects, however, were found by Lupien et al. (1999) or Fehm-Wolfsdorf et al. (1993) following the administration of higher doses of hydrocortisone (i.e., 16.6 mg and 50 mg respectively). Newcomer et al. (1999) also reported a similar lack of effects on declarative memory following the administration of 40 mg hydrocortisone. Moreover, as they did identify detrimental effects following the administration of 160 mg, they interpreted these results as suggesting that only severe levels of stress may be detrimental to declarative memory. As the results of this

current study also found no effects of acute increases in cortisol levels on declarative memory, these results may be interpreted in a similar way.

There are, however, other potential explanations which may explain this lack of effects. For example, the lack of effects following acute changes in cortisol levels could point to a methodological problem. As described in Chapter 1, even the most subtle differences in methodologies can be used to explain discrepancies in results between studies, and some of the same methodological explanations used by previous researchers can be used to explain the discrepancies in results obtained here. For example, Lupien et al. (1999) suggested that the discrepancy in results between their study and those found by Kirschbaum et al. may have been an effect of using different types of encoding instructions. Lupien et al., like the current study, used intentional encoding. In contrast, Kirschbaum et al. used incidental encoding. Intentional encoding is when participants are made aware at the time of learning that the information they are about to receive will have to be recalled later on. In contrast, incidental encoding is when participants are not primed to remember. According to Mandler (1967), in comparison to incidental encoding, intentional encoding can lead to higher recall performance.

A second potential methodological explanation relates to the specific type of memory tasks which were used. Again, this is a similar explanation to that previously put forward by Lupien et al. (1999). As described in Chapter 1, the declarative memory task used by Lupien et al. was designed specifically not to overload the 'limited processing capacity of working memory (Lupien et al., 1999, p. 427), consequently it was much shorter than that used by Kirschbaum et al. (1996). The declarative memory task used in this current

study was also very short. Consequently, this implies that the results might have been different if a longer declarative task had been used. Incorporating a delay between learning and recall also gives greater assurance that long-term memory is being assessed (Wolkowitz et al., 1997); this explanation was put forward by Lupien & McEwen (1997) to explain the discrepancy in results between Kirschbaum et al. and Beckwith et al. (1986). Like Beckwith et al., there was no delay between learning and testing in this current study, which may explain the lack of effects of acute changes in cortisol levels on declarative memory.

In addition to these potential explanations, the results of a more recently study by De Quervain et al. (2000) suggests that the effects of acute changes in cortisol levels on declarative memory are influenced by the timing of treatment relative to learning and testing. As described in Chapter 1, according to these researchers, the acute administration of hydrocortisone either pre-learning or immediately post-learning has no effects on either recall or recognition performance; this is because of its specific effects on the retrieval phase of declarative memory. Like De Quervain et al., in this current study similar levels of hydrocortisone were administered to participants one hour prior to learning and testing. Consequently, according to this interpretation, the results produced were in the expected direction.

In summary, therefore, the results of previous research point to several possible methodological explanations for the lack of effects of acute changes in cortisol levels on declarative memory identified by this current study. Indeed, the results of this current study have highlighted the importance of considering differences in methodologies when comparing results.

Notwithstanding this, however, previous researchers have also identified similar dissociations between acute changes in cortisol levels and declarative memory (e.g., Lupien et al., 1999; Newcomer et al., 1999). Consequently, the results of this current study may simply lend support to the claims that the effects on declarative memory produced following chronic changes in cortisol levels are, indeed, very different to those produced following acute changes.

3.6.2. *Effects of acute changes in cortisol levels on working memory*

Whilst the results of this current study support the lack of effects on declarative memory identified by Lupien et al. (1999), they did not find the same detrimental effects following acute changes in cortisol levels (both high and low) on working memory. Moreover, as both studies used the same working memory item-recognition task, which also suggested that both groups of participants were using the same type of search strategies (discussed in Chapter 5), this further suggests that the discrepancy in results did not point to a methodological problem.

There is, however, one methodological difference between the two studies which might, potentially, explain the discrepancy in results. This relates to the method of administration of cortisol. As described in Chapter 1, Lupien et al. infused varying dosages of hydrocortisone for a total of 100 minutes, starting from 45 minutes prior to testing and continuously throughout. In contrast, the doses of hydrocortisone in the current study were administered orally, in tablet form. Participants were instructed to self-administer the tablets from up to two to three hours prior to testing, with the last tablet being taken one hour before testing was carried out. Consequently,

the method used to administer hydrocortisone may have influenced the results. In addition, making participants responsible for administering their own tablets and at set times introduces the issue of compliance. If not present during this time, investigators can only trust that their participants administer their medication as instructed.

There are, however, two potential problems with this interpretation of the results. First, previous studies reporting similar detrimental effects following chronic changes in cortisol on declarative memory have also used different methods of administration. Second, the levels of cortisol produced by participants in this current study following the oral administration hydrocortisone (i.e., 30 mg and 10 mg) were comparable to those produced following the administration of hydrocortisone by infusion (i.e., approximately 8.3 mg and 16.6 mg). Specifically, participants produced mean salivary cortisol levels of 323.74 nMols/L and 81.44 nMols /L following the administration of 30 mg and 10 mg hydrocortisone respectively, compared to between 53.7-66.10 nMols/L and 89.75-111.30 nMols/L salivary cortisol following the administration of 8.3 mg and 16.6 mg hydrocortisone respectively. Consequently, these results suggest that this discrepancy in results may not be explained by the method of cortisol administration alone.

In addition to reaction times, the item-recognition task used in both studies recorded the numbers of detection errors that participants had made. Like Lupien et al., the percentage of detection errors in this current study was very low (i.e., between 0 and 1.71%). As predicted, there was also a significant positive relationship between the number of detection errors and comparison load. This supports the prediction that as the number of

comparison loads increase a task becomes harder. In all three conditions, the percentages of detection errors were also higher after comparison load 8. This might have been predicted in relation to Miller's 7 ± 2 item capacity for working memory (i.e., he found that the processing capacity of working memory store in normal young adults is approximately between 5 and 9 items; Miller, 1956). Taken together, therefore, this suggests that the discrepancy in effects on working memory do not point to a methodological problem. Moreover, until more studies identify similar results to Lupien et al., it still remains questionable whether working memory is more sensitive to the detrimental effects of acute changes in cortisol than declarative memory.

3.6.3. Time of day effects on memory performance

In addition to the effects of acute changes in cortisol levels, the current study also investigated whether any effects of cortisol on memory performance could be explained by the time of day. Moreover, as Fehm-Wolfsdorf et al. (1993) had also investigated the effects of time of day, these two sets of results were compared. As mentioned previously, the effects of acute changes in cortisol levels were investigated in this current study at either 09.00 or 10.00 hrs (depending on condition) and at 17.00 hrs. However, whereas Fehm-Wolfsdorf et al. found that participants in their control condition performed better in the morning compared to the evening (i.e., at 09.00 hrs vs. 18.00 hrs respectively), no differences were found as a function of time of day in the control group in the current study. Notwithstanding this, the current study found significant and positive relationships between morning cortisol levels in the control group and performance levels in: (1) Hopkin's recall performance;

and (2) performance in the Doors task. This suggests that higher endogenous cortisol levels in the morning facilitate this aspect of memory. They also found a significant and negative relationship between afternoon cortisol levels in the high cortisol group and Speed of Processing performance in the afternoon. This suggests that higher endogenous cortisol levels in the afternoon impair this aspect of memory. Taken together, therefore, these results suggest that a relationship between time of day and effects of cortisol levels on memory performance do exist.

3.6.4. Perceived levels of stress and salivary cortisol

As described previously, the results of this current study showed a significant difference between the mean perceived levels of stress for each condition. Moreover, these differences in levels were in the predicted direction, with participants in the high cortisol condition reporting the highest perceived levels of stress overall (although they were higher in the afternoon than in the morning), participants control condition reporting higher levels in the morning compared to the afternoon, and participants in the low cortisol condition reporting similar levels at both times of day. As the levels of cortisol for participants in the high and low cortisol conditions were manipulated pharmacologically, this suggests that an individual's perception of stress may be influenced by their cortisol levels.

As described in Chapter 1, however, the results suggesting a relationship between perceived levels of stress and cortisol levels are mixed. For example, although Lupien et al. (1998) identified a significant and positive relationship between perceived levels of stress and salivary cortisol levels. De

Quervain et al. (2000) found no such relationship one hour following the administration of 25 mg hydrocortisone. Vedhara et al. (2000) also found that, although perceived levels of stress increased in students during exams, this was associated with a significant reduction in salivary cortisol levels. The results of this current study found a significant and positive relationship between salivary cortisol levels and perceived levels of stress in the afternoon, with participants who produced the highest levels of cortisol also reporting the highest levels of perceived stress. Moreover, although this relationship was not significant in the morning, it was only just non-significant at $p = 0.06$. This implies that the relationship may have been significant with a larger sample size. Taken together, therefore, the results of this current study do suggest a relationship between an individual's perception of stress and cortisol levels, although the cause of such an effect is unclear (i.e., does an individual's levels of cortisol influence their perceived levels of stress or vice versa).

3.6.5. New research findings

In addition to producing results for comparison with previous research, the current study was designed to investigate some new areas of research. These included the effects low levels of cortisol on memory performance, and the effects of acute changes in cortisol levels on the episodic and semantic components of declarative memory. The results pertaining to these issues are discussed.

- *Effects of low levels of cortisol*

As mentioned previously, the rationale for having a low cortisol condition was to investigate the effect of minimal levels of cortisol on memory performance which, at the time of writing this thesis, did not appear to have been examined before. To do this, 1500 mg metyrapone (a cortisol-synthesis inhibitor) was administered to participants at each of two times of day to see if any differences in the effects on memory performance as a function of cortisol levels or time of day occurred. However, whilst the results of the current study showed clearly significant differences in salivary cortisol levels three hours following the administration metyrapone compared to those produced by participants in the high cortisol and control conditions, they found no significant effects on memory performance and at both times of day. They also showed that the reduction in cortisol levels produced following the administration of the same doses of metyrapone at two times of day depended on the basal levels of cortisol at that time. More specifically, with mean salivary cortisol levels of 4.68 vs. 2.34 nMols/L for the morning vs. afternoon respectively, the morning levels of participants in the low cortisol condition were exactly 100% higher than those observed in the afternoon. This shows that, although metyrapone was effective in suppressing cortisol secretory activity, a circadian pattern was still detectable.

- *Effects of acute changes in cortisol levels on the episodic and semantic components of declarative memory*

Although previous studies have looked at the different components of declarative memory, it is not clear whether the effects of acute changes in

cortisol levels affect the episodic and semantic components to the same degree, or whether episodic memory is affected to a greater degree than semantic memory. As described in Chapter 1, clarification of this uncertainty would provide further insight into whether the episodic and semantic components of declarative memory are both dependant, and if so to what degree, on the integrity of the hippocampus.

The episodic and semantic components of declarative memory were examined using a range of episodic and semantic memory tasks. However, whilst some previous research using the same types of tasks has reported detrimental effects of cortisol on declarative memory, the results of the current study failed to find any significant effects of acute changes in cortisol levels on either component at either time of day. Indeed, the performance levels for the episodic and semantic memory tasks were similar for all three groups and at both times of day.

On one hand, this lack of effects might be explained by the cognitive measures used (e.g., the HVLT). In the current study, participants in each condition and at both times of day produced recognition scores at ceiling level (i.e., 12). This suggests that the HVLT task may have been too easy for the participants and/or not 'arousing' enough. Indeed, De Quervain et al. (2000) attributed the lack of any memory enhancing effects of hydrocortisone post-learning to the non-arousing learning conditions. Although hydrocortisone was administered prior to training in this current study, the same explanation may be implicated here.

One of the reasons for using the HVLT, apart from it being a reliable and valid measure of episodic memory, is that it is also a very short task

comprising a total of twelve words (i.e., three categories of four words). As participants in this current study were required to complete a battery of ten individual memory tasks, it was decided that shorter tasks should be used to avoid any fatigue effects. Lupien et al. (1999) used a short declarative memory task in their study (i.e., it comprised twelve pairs of word), as they wanted a task 'designed specifically not to overload the limited processing capacity of working memory system'. Indeed, Lupien et al. even suggested that the reason Kirschbaum et al. (1996) identified impaired declarative memory recall in their study was because the task they used had a 'high processing demand at the time of encoding or during consolidation'. The declarative memory task used by Kirschbaum et al. comprised a twenty-six word task, together with a spatial memory task which required active processing (as opposed to pure storage) whereby participants had to mentally rotate a spatial map before recall.

In addition to using a longer task, Kirschbaum et al. (1996) also incorporated a delay between learning and recall in their study. As described previously, this more fully assures that long-term memory is being assessed. Kirschbaum et al. reported detrimental effects of cortisol on declarative memory using this procedure, however, when the same protocol was used initially but with no delay (i.e., by Beckwith et al., 1986), no detrimental effects on declarative memory were found. Consequently, as there was no delay between learning and testing in this current study, this suggests that the assessment of long-term memory may have been inadequate.

In addition to no effects on recall performance, the results of this current study also failed to identify any significant effects of cortisol on

recognition performance using the HVLT. Again, this may reflect that acute changes in cortisol levels do not affect recognition memory. Indeed, De Quervain et al. (2000) did not identify any effects on recognition memory following chronic changes in cortisol levels, although Wolkowitz et al. (1990) did identify impaired recognition performance following the administration of chronic levels of prednisone. Again, it is important to note that both of these studies investigated the effects of chronic, not acute, changes in cortisol levels. They also used different types of steroids (i.e., hydrocortisone vs. prednisone) and, as the rate of absorption by the brain is different for different steroids (e.g., Coirini et al., 1994; Meijer et al., 1998) this may have modified the results produced.

Two different, but comparable versions of the Doors and Names tasks were also used to measure the effects of acute changes in cortisol levels on episodic memory. However, both tasks identified no effects on memory performance as a function of corticosteroids or times of day. As there were also no significant differences between the mean scores for the Names and the Doors tasks, this suggests that acute changes in cortisol levels do not affect either the verbal or visual aspects of recognition memory.

The semantic memory tasks used in this current study comprised the Speed of Processing task and the Spot the Word task. Although there were no significant differences in speed of processing scores as a function of cortisol levels, in contrast to the episodic memory tasks, there was a significant difference between these scores as a function of time of day ($F = 5.472$; $df = 1.000$; $p < 0.05$). Specifically, participants performed better in the afternoon compared to the morning. This may be explained by the effects of arousal

levels which, by being higher in the afternoon compared to the morning, have been shown to enhance declarative memory (Folkard & Monk, 1979). Notwithstanding this, however, the results of this study did not show any effects of acute changes in cortisol levels in either episodic or semantic memory performance. Consequently, it is still unclear whether both components of declarative memory are dependent upon the integrity of the hippocampus when cortisol levels change.

3.6.6. Other observations

In addition to the results described above, perhaps the most significant findings be produced by this current study relate to the individual differences in cortisol-response and, more importantly, how even when significant differences in cortisol levels were found, there were no differences in the levels of memory performance. These results, together with the potential effects of the target population, are now discussed.

- *Individual differences in cortisol-response*

Chapter 2 described how individual differences in cortisol-response have been identified by previous researchers (Bohnen et al., 1990; Kirschbaum et al., 1996; and Lupien et al., 1997) and, like these studies, individual differences in cortisol-response were also identified in this cortisol study. Indeed, significant individual differences in mean cortisol-levels were produced in all three groups irrespective of whether the acute changes in cortisol levels were manipulated using medication (i.e., in the high and low cortisol groups), or as a result of time of day (i.e., in the control group). Moreover, the range of

cortisol-responses produced by participants within each group enabled a clear and significant distinction between high- and low-cortisol responders to be made. High cortisol-responders were categorised as those participants who produced cortisol levels above the condition mean, whereas low cortisol-responders were categorised as those who produced cortisol levels below the mean.

One explanation for individual differences in cortisol-response to medication (i.e., hydrocortisone and metyrapone) may be related to individual differences in response to chemicals and the rates at which these chemicals are absorbed into the system. These rates of absorption can also be influenced by other factors, such as whether food has just been consumed (e.g., Brutsche, Brutsche, & Munawar, 2000). As the participants were asked to eat their 'normal' meals prior to testing, including not having to eat anything, this highlights a potential weakness in the design of this study. Specifically that perhaps participants should have been asked to eat something to control for rates of medication absorption. Individual differences in cortisol-response to changes in cortisol levels which are produced naturally, however, have been explained differently. Brown et al. (1996) suggested that individual differences in the cortisol-response may be explained by differences in anxiety levels. As described in Chapter 2, Brown et al. found that repressors and high-anxious participants demonstrated higher basal salivary cortisol levels compared to low-anxious participants; they did not, however, examine these effects on memory performance. A comparison of anxiety levels (i.e., GHQ scores) with salivary cortisol levels was also carried out in this current study. In support of Brown et al., these showed highly significant and positive

relationships between GHQ scores and salivary cortisol levels at both times of day (i.e., $r = 0.410$; $p = 0.001$ vs. $r = 0.298$; $p < 0.05$ for morning vs. afternoon levels respectively). Consequently, the results of this current study go some way to suggest that an individual's cortisol-response may be positively related to their anxiety levels.

However, as well as identifying individual differences between high- and low-cortisol responders following chronic changes in cortisol levels, Bohnen et al., Kirschbaum et al., and Lupien et al. also found that the high-cortisol responders showed poorer declarative memory compared to the low-cortisol responders. No such differences in effects on memory performance between the high- and low-cortisol responders were found in this current study. Moreover, apart from a significant between-group difference in Speed of Processing scores in the low-cortisol responders in the afternoon, a post-hoc comparison of memory scores between the high- and low-cortisol responders in each group and at both times of day showed no other significant differences. The results did, however, show a consistent pattern in cortisol-response behaviour. Specifically, that a high responder in the morning tended to be a high responder in the afternoon, and vice versa. The results of this current study, therefore, imply that it may not be the levels of cortisol per se. that determine the effects on memory performance. Moreover, that the effects may depend on how the individual perceives and/or copes with the effects of changes in cortisol. For example, the effects produced may be moderated by personality and/or experiential factors, as suggested by previous researchers (e.g., Pruessner et al., 1999; Schaubroeck et al., 2001).

- *Effects of target population*

One such experiential factor is prior experience of stress (i.e., if the individual has become habituated to the effects of stress). Chapter 1 described how habituation to stress can occur in some individuals following repeated exposure to certain stressors (Gerra et al., 2001). It also described how examination stress is deemed a 'predictable and often recurring stressor' in students, as an explanation for why the cortisol levels of a group of students at baseline did not increase during exams students (Vedhara et al., 2000). The participants in this study were students. As students are used to experiencing feelings of stress, the lack of effects of acute changes in cortisol levels on their memory performance may be explained by habituation.

There is one potential problem, however, with this interpretation of results. If the participants in this current study had, indeed, become habituated to stress, then it could be predicted that this would be reflected by their perceived levels of stress (i.e., their perceived levels of stress would be similar across all three conditions as well as at both times of day). For example, a participant in the stress condition who had become habituated to feeling stressed would be expected to report a similar perceived level of stress as a participant in the control condition. As described previously, however, the results of this current study did show significant differences in perceived levels of stress between the three groups (i.e., $F(2,57) = 4.536$; $p < 0.05$).

A second potential problem with explaining the dissociation between the effects of cortisol and memory performance by the type of target population relates to the findings made by Schmidt et al. (1999). Schmidt et al. carried out their study using students and they did find significant effects

on declarative memory as a function of cortisol levels. However, as they were also interested in the delayed effects of cortisol, in contrast to this current study, they incorporated a delay between learning and testing, which more fully assures that the effects of changes in cortisol levels on declarative memory are being tested.

3.6.7. *Conclusions and way forward*

To summarise, therefore, differences in methodology appear to be one very significant and potential explanation for the discrepancy in results between this current study and those identified by previous research. These include differences between: chronic versus acute changes in cortisol; time between learning and testing; method of administration of method (i.e., tablets vs. intravenous); type of encoding instructions; and target population of participants. Many previous studies which have identified detrimental effects of changes in cortisol levels on declarative memory have also been those which have used elderly and clinical populations. Consequently, their findings may have been affected by other factors relating to age and/or pathology. This makes comparison of the results of this study difficult to do.

Despite these methodological differences, however, it can be concluded that this current study has made some contribution to our understanding of the effects of acute changes in cortisol levels on memory in five different ways. First, as very few studies have investigated the effects of acute changes in cortisol, these results lend further support to the claim made by Newcomer et al. (1999) that it may only be extreme levels of stress, or long-term exposure to lower levels of stress (e.g., following long-term

treatment with steroids), that effect memory performance. Second, they offer some insight into the effects of acute changes of low levels of cortisol on memory performance, which had not been investigated before (i.e., there was no difference in effects compared to those produced following high and normal changes in cortisol levels). Third, they provide some insight into the effects of acute changes in cortisol levels on the episodic and semantic components of declarative memory (i.e., there was no difference in effects on either episodic or semantic memory). Fourth, they highlight individual differences in cortisol-response, both to endogenously and exogenously induced manipulations in cortisol levels. However, fifth, and possibly the most significant contribution made by this current study, is how even with significant differences in salivary cortisol levels (i.e., between conditions and between high- and low-cortisol responders within each condition), there were no differences in either aspect of memory performance at both times of day. Taken together, therefore, the overall results of this study suggest that it may not be the levels of cortisol per se. which explain the effects on memory performance identified by previous researchers, and that these effects may be attributed to other factors (e.g., personality, situation, baseline levels of cortisol, mood, or the individual's cognitive appraisal). What future researchers need to explore are what these factors may be. In addition, even though there was no difference in effects on memory performance, from a health-related perspective future researchers also need to pay some attention towards what makes one individual a high-cortisol responder and another a low-cortisol responder. One approach to this question could be to identify the factors that stimulate cortisol secretion that might be shared by high-cortisol

responders, and differentiate these from those identified in low-cortisol responders. The reasons for individual differences in cortisol response may be beyond the scope of psychology alone (e.g., neuroendocrinology or genetics). However, it is only by understanding these differences and those factors which may make one individual more vulnerable to the effects of cortisol than another, that will enable preventative and remedial measures to be identified.

In conclusion, therefore, there is still plenty of scope for further studies to be carried out investigating the effects of acute changes in cortisol levels on memory performance. Indeed, these need to be carried out in order to identify how much stress needs to be present before memory suffers, and whether short periods of high levels of cortisol are more detrimental than longer periods of lower levels of cortisol on memory. The part played by the individual (i.e., personality and situational), together with individual differences in cortisol-response, also need greater consideration in the designs that are used.

4. Experiment 3 : The Addison's patient study

4.1. Abstract

The mineralocorticoid (MRs) and glucocorticoid (GRs) receptors are found in abundance in the frontal and hippocampal regions of the brain. Both types of receptors are activated under normal basal cortisol levels, however there is some evidence to show that increased occupancy of the GRs, by high levels of circulating cortisol, may result in memory deficits. Using a repeated measures design with nine patients with Addison's disease (mean age = 38.3 years), the immediate effects (i.e., with no delay between learning and testing) following activation of the MRs only, GRs only, and both types of receptors were investigated on working memory and the episodic and semantic components of declarative memory. The different receptors were activated using steroids. As predicted, the results showed that participants produced poorer working memory performance (using the digits backward task) when the GRs only were activated ($p<0.05$). In contrast, they showed poorer episodic memory performance (using the Hopkins Verbal Learning recall task) when the MRs only were activated ($p<0.05$). During both tasks, participants produced the best scores when both receptors were activated. Whilst the significant effects identified following activation of the different receptors were not consistent across all memory tasks, the results do suggest that individuals show better memory performance when both receptors are activated. This supports the suggestion that balanced activation of MRs and GRs is necessary for optimal memory function. The results also support previous studies in rats showing that activation of the MRs is essential during sensory storage (i.e., encoding) whereas activation of the GRs (in addition to the already activated MRs) is essential during memory consolidation and retrieval.

4.2. Introduction

Chapter 1 described how one explanation for the selective effects of cortisol on memory relates to the organisation of memory in the brain and the availability of the MRs and GRs, the two types of corticosteroid receptors. The MRs and GRs are located throughout the brain, however, they are particularly abundant in the hippocampus and frontal lobes. Consequently, this explains why it is the memory functions dependent on the integrity of these areas of the brain (i.e., declarative memory and working memory respectively) which are sensitive to the effects of cortisol.

Chapter 1 also described how the corticosteroid receptors are activated by different levels of cortisol and that this relates to a tenfold difference in affinity levels (De Kloet, 1991). Normal basal levels of cortisol are sufficient for occupancy of the MRs, whereas only increased levels of cortisol (such as those produced during stress) activate the GRs and the already activated MRs. However, although moderate activation of the GRs appears to be a pre-requisite for long-term memory (De Quervain et al., 1998), prolonged exposure to increased activation of the GRs can be harmful. Consequently, it has been suggested that the detrimental effects of cortisol on memory are sustained by increased activation of the GRs (e.g., Bremner et al., 1995; Mauri et al., 1993; Simmons et al., 2000).

Chapter 1 also described how previous research in non-primates suggests that activation of the MRs and GRs affect different aspects of information processing (e.g., Oitzl & De Kloet, 1992); there is currently no research investigating this in humans. Oitzl & De Kloet were the first researchers to investigate the effects of corticosteroids on the different phases of memory formation using MR and GR antagonists (i.e., the compounds RU28318 and RU38486 respectively) in rats. These

antagonists were given to one group of rats either before training or after training the Morris water maze task for the first time, and in a second group of rats using the same protocol for the second time. The results showed that the administration of the GR antagonist (which interferes with GR activation) before and after training impaired the rats' spatial navigation in the Morris Water Maze task after the first training/testing session, but not after a second one. More specifically, Oitzl & De Kloet showed that the administration of the GR antagonist impaired the rats during the acquisition and/or consolidation phases of memory. Conversely, when the rats were injected, using the same protocol, with an MR antagonist (which interferes with MR activation), this had no effect during acquisition and/or consolidation. Consequently, this led the researchers to conclude that the MR activation 'is involved in behavioural reactivity in response to environmental cues', whereas the GR-mediated effects 'promote consolidation of acquired information' (Lupien et al., 2002, p.412).

The purpose of Chapter 4, therefore, is to describe the details of an experiment which was carried out on a group of patients with Addison's disease to investigate whether prolonged exposure to high levels of corticosteroids has detrimental effects on memory. The principle aim was to investigate whether balanced activation of both the MRs and GRs is necessary for optimal memory function and whether, as previously identified in non-primates, activation of the MRs and GRs affect different aspects of information processing in humans. However, before doing this, the next section will briefly describe Addison's disease and describe the rationale for using Addison's patients in this study.

4.2.1. Addison's disease

Dr Thomas Addison first identified Addison's disease in 1849. This is a rare hormonal disorder that affects about 1 in 100,000 people and occurs in all age groups, afflicting men and women equally. The disease is characterised by weight loss, muscle weakness, fatigue, low blood pressure and, sometimes by hyperpigmentation (i.e., a darkening of the skin) in both exposed and non-exposed parts of the body. Indeed, in the early stages of diagnosis, it is usually this hyperpigmentation that may lead a doctor to first suspect Addison's disease.

Addison's disease occurs when the adrenal glands do not produce enough cortisol and, in some cases, the additional hormone, aldosterone. For this reason, therefore, the disease is also known as chronic adrenal insufficiency, or hypocortisolism. Consequently, because cortisol is so important for health, most significantly by helping the body respond to stress, patients with Addison's disease are given treatments which involve replacing, or substituting, the hormones that the adrenal glands are not making. This replacement is carried out using steroids. Cortisol is normally replaced orally with hydrocortisone or prednisone (which activates both MRs and GRs) plus an additional mineralocorticoid (e.g., fludrocortisone) to control the body's sodium and potassium needs and keeps the blood pressure normal. If aldosterone is also deficient, this is replaced with oral doses of fludrocortisone acetate.

Identifying the correct dose of steroids for the treatment of Addison's disease depends on the individual and, in some cases, may be the result of a process of trial and error. The dose applied will not be as high as the high

doses of powerful steroids that may be given to patients with diseases such as rheumatoid arthritis. Rather, it will be sufficient enough to provide the lowest level of replacement that will allow the patient to feel well and have a normal life but without the problems of overdose. Moreover, the doses of steroids given are also designed to imitate the normal daily rhythm of cortisol secretion. This means that a relatively larger dose is given first thing in the morning followed by a smaller dose in the afternoon. As some patients feel less well before their second dose, these patients can benefit from splitting the daily dose into three (i.e., administered in the morning, midday and early evening). During a stressful situation, a doctor may also suggest a temporary increase in dose.

The symptoms of Addison's disease progress slowly and, consequently, the early symptoms are often ignored until a stressful event, like an illness or an accident, causes them to become worse. This is often referred to as an addisonian crisis. The symptoms for an addisonian crisis can include sudden penetrating pain in the lower back, abdomen, or legs; severe vomiting and diarrhea, followed by dehydration; low blood pressure; and loss of consciousness. Left untreated, an addisonian crisis can also be fatal. Fortunately, however, in most patients the symptoms are severe enough to urge the individual to seek medical treatment before a crisis occurs (e.g., chronic fatigue and severe lethargy).

4.2.2. Rationale for using Addison's patients

The rationale for using Addison's patients in this study, therefore, was twofold. First, as Addison's patients do not produce sufficient levels of

cortisol endogenously, they provide a target population in which the two types of corticosteroid receptors can be activated separately, using different steroids. In this study, fludrocortisone was used to activate MRs only and dexamethasone was used to activate GRs only. By activating the different corticosteroid receptors individually as well as together, this provided three different activation conditions (i.e., activation of the: MRs only, GRs only and MRs/GRs) under which the effects of cortisol on memory performance were measured. It also provided an opportunity to investigate whether activation of the MRs and GRs in humans (as previously identified in rats) affect different aspects of memory performance. Moreover, as the levels of cortisol were replaced using steroids and the administration of steroids suppresses the circadian variation in cortisol release (Fehm-Wolfsdorf et al., 1993), testing was only carried out at one time of day (i.e., at 11.00 hrs).

Second, as Addison's patients are treated with replacement levels of cortisol throughout life, this population provided an opportunity to investigate the effects of chronic treatment with steroids on memory performance. It also provided an opportunity to further investigate the relationship between duration of treatment and memory performance. As described in Chapter 1, Keenan et al. (1995) identified a significant age by duration effect between younger and older participants (i.e., those who were younger than 45 years compared to those who were older than 45 years) who had received long-term treatment with prednisone. They found that, although both groups showed impaired declarative memory performance, the detrimental effects on memory appeared to plateau after the first three years of treatment in the older-aged participants (i.e., in those who were younger than 45 years). As these results

have not been replicated, this data would provide further insight into the effects of chronic treatment with steroids on memory performance.

- *Problems associated with using Addison's patients*

Although there were several benefits with using Addison's patients, there were also several potential problems. First, as described in Chapter 1, a study using patients can make it difficult to discriminate the effects of cortisol on memory performance from those brought about by the pathology alone (Wolkowitz et al., 1990).

Second, as Addison's disease is very rare, this raised concerns over the availability of participants for recruitment. As a consequence of this, therefore, the exclusion/inclusion criteria applied in Experiment 2 was made more flexible for this study (i.e., participants were still recruited into the study if they were smokers; had BDI scores > 11; had GHQ scores > 60; and had any family history of serious illness. In this current study, none of the participants were smokers. Moreover, as Addison's disease affects men and women equally, females were also recruited into the study. As previous research has identified an effect of gender on cortisol levels and memory performance (e.g., Seeman et al., 1997 found that females with high levels of cortisol showed the poorer declarative memory performance), all females who were recruited into the study and were still menstruating at this time, were tested between days 5 and 11 of their luteal phase; in this current study only two participants fulfilled this criteria.

Third, as each of the three conditions in the current study involved participants adapting their normal medication regimes for 48 hours prior to

each testing session, this raised concerns as to how participants would feel about this. More specifically, would it discourage them from taking part. To address this, therefore, all patients who were invited to take part in the study were those currently being treated by Professor Lightman (co-supervisor of this research and a senior endocrinologist) or by one of his colleagues at the BRI. Second, all volunteers who showed an interest in taking part were invited to discuss the study with the researcher and Professor Lightman beforehand. Third, a letter was sent out to each patient's general practitioner (GP) asking if there were any reasons why they did not feel that their patient should take part. A copy of the testing procedure and important information was also sent to the patient's GP. Fourth, the study was only carried out after receiving the full ethical approval of the United Bristol Healthcare NHS Trust and the adequate understanding and written consent of all participants (see Appendix III for copy of consent form).

In addition to the controls described above, the study was also carried out using a repeated measures design. Consequently, as this meant every patient acted as their own control, any potential effects from other factors were consistent over all three testing sessions.

4.3. The current study

4.3.1. Aims of the study

The primary aims of the current study, therefore, were two-fold. The first aim was to compare the effects of cortisol on memory performance following activation of : MRs only; GRs only; and a combination of MRs/GRs. Fludrocortisone was used to activate MRs only, dexamethasone was used to

activate GRs only, and a combination of both steroids was used to activate the MRs/GRs.

The second aim of the current study was to investigate the effects of acute changes (i.e., 48 hours) in receptor activation on working memory and the episodic and semantic components of declarative memory, and in a population who had received chronic levels of treatment with steroids. Whilst the effects of chronic changes in cortisol levels on declarative memory have been explored, the effects of chronic changes in cortisol levels on working memory are less clear.

4.3.2. Hypotheses

Based on the above, therefore, the following predictions were made:

Activation of the MRs only will affect declarative memory performance, whereas activation of the GRs only will affect working memory performance. This was based on research carried out by Oitzl & De Kloet (1992) using rats.

In contrast to activation of MRs only or GRs only, participants will show better memory performance when both corticosteroid receptors are activated. This was based on the claim that balanced occupation of both receptors is necessary for optimal memory function (De Kloet et al., 1998).

There will be a relationship between duration of treatment with steroids and degree of memory performance.

4.4. Methods

4.4.1. Design

A repeated measures design was used in which nine participants were allocated to each one of three within-group conditions. These conditions were based on which type of steroids participants received and comprised: (1) MRs only (0.2 mg fludrocortisone/day); (2) GRs only (1.0 mg dexamethasone/ day) and (3) MRs/GRs (a combination of 0.2 mg fludrocortisone and 1.0 mg dexamethasone/day). The rationale for the doses of steroid administered was to provide a high level of occupancy of GRs only, MRs only, and MRs/GRs and were agreed by Professor Lightman.

The order of testing sessions was randomised by the researcher by pseudo-randomisation. This was carried out using each of the six possible combinations of session order (i.e., 1-2-3; 3-1-2; 2-3-1; 2-1-3; 1-3-2; and 3-2-1), which were written down on separate pieces of paper and placed in a bag. As each participant was recruited into the study, they were asked to withdraw a label from the bag to identify their order of sessions; all participants were blind to what each session comprised. After the last label was withdrawn, all six labels were then replaced so that three labels could be withdrawn for the final three participants. This meant that at least one participant carried out a different session order and that three participants carried out the same session orders. The dependent variable was memory performance and this was measured using three different batteries of memory tasks designed to test the effects of steroids on working memory, episodic memory and semantic memory. The order of each battery was also randomised by the researcher using the same procedure used for the testing sessions. Other measures

obtained during the first testing session included: age; sex; BMI; IQ score; number of years with Addison's disease; and details of other pathologies together with current medication. Measures obtained at each testing session included: perceived levels of stress; approximate caffeine intake from 24 hours prior to testing; types of food-group eaten prior to testing; serum cortisol levels; and glucose levels. All blood samples were taken intravenously by a qualified nurse in the BRI at the end of each testing session.

The effects of cortisol on memory performance were tested at 11.00 hrs on each day of testing. A period of one month was allowed between each testing session. This was to allow any effects brought about by the different types of medication, to return to normal. During this period, participants were told to continue with their normal regime until two days prior to the next testing session, when the regime would be changed. Any females who were still menstruating during the testing period were tested between days 5 and 11 of their luteal phase only. This was to control for the additional effects of oestrogen on memory performance. In addition, only one participant was tested during each testing session.

4.4.2. Participants

A total of eleven participants were initially recruited into the study. However, two participants failed to complete all three testing conditions due to an adverse health reaction to the MRs only condition. Unfortunately, during the MRs only condition, the patients are GR deficient and one of the problems associated with this deficiency is that patients may feel lethargic and generally less well than normal. Consequently, although this reaction was not

anticipated, it was not too surprising. The data for a total of nine participants only (i.e., four females and five males), therefore, was used in this current study.

With a total of nine participants who each had to complete three conditions, it was calculated that this would produce statistical power effect sizes of : 0.71 for working memory; 0.91 for episodic memory; and 0.99 for semantic memory. The effect sizes for the declarative aspects of memory performance were high because of the high between-level correlations found between the scores. Full details of the calculations produced by the analysis of these results can be found in Appendix XXVI.

Table XXI shows the mean age, body mass index (BMI), depression (BDI), anxiety (GHQ) and IQ (NART) scores for the nine participants. This shows very little difference in each of these measures between males and females. Indeed, a series of independent samples t-tests showed that there was no significant differences between males and females in age ($t = -1.371$; $df = 7$; NS) and BMI ($t = 0.196$; $df = 7$; NS). There was also very little difference between the males and females on scores for: IQ ($t = -2.176$; $df = 7$; NS); BDI ($t = 0.290$; $df = 7$; NS) and GHQ ($t = 0.314$; $df = 7$; NS).

Table XXI : Mean scores for age, BMI, BDI, GHQ and NART (N = 9)

	Total group N = 9		**Males only N = 5**		**Females only N = 4**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (years)	37.90	9.51	34.20	9.58	42.50	8.23
BMI	26.02	5.79	23.70	3.52	28.93	7.26
BDI	4.22	4.12	4.60	3.05	3.75	5.68
GHQ	52.89	7.85	55.40	8.73	49.75	6.24
IQ	111.00	13.07	104.00	11.20	119.75	10.21

Participants were also asked to report the details of any other illnesses (e.g., chronic inflammatory disease, psychiatric disorders, obesity, coronary heart disease, sleep disorders, diabetes or any other 'abnormal' glucose condition, and any other serious medical condition) and whether there was any history of serious family illness. They were also asked to provide details of any other medication they were receiving. Four of the nine participants had been diagnosed with other illnesses. Moreover, these participants were all female.

The participants were also asked to provide details of their normal medication regime for Addison's disease. This showed that all participants were currently being treated for Addison's disease with a combination of hydrocortisone and fludrocortisone. They were also asked to state the number of years since diagnosis with Addison's disease; this ranged from 5 – 27 years.

4.4.3. *Materials/Apparatus*

The following quantities are per participant (N = 9).

- Letter sent out to Addison's patients asking for volunteers for study (see Appendix XVI)
- Letter sent out to Addison's patients with details of initial meeting to discuss study (see Appendix XVII).
- Information Sheet given to Addison's patients at initial information meeting (see Appendix XVIII)
- Letter sent out to each Addison's patients' GP (Appendix XIX)
- Letter sent to volunteers with selection of dates for testing (Appendix XX)

- Letter sent to volunteers with confirmed date of first testing session, tablets and instructions for administration (see Appendix XXI).
- Instructions for subsequent testing sessions (see Appendix XXII)
- Personal Record Sheet (see Appendix VI)
- Consent Form (see Appendix III)
- Beck Depression Inventory (BDI-21 – see Appendix VII)
- General Health Questionnaire (GHQ-30 – see Appendix VIII)
- National Adult Reading Test (NART – see Appendix XXIV)
- Medication, i.e., fludrocortisone (12 x 0.1 mg tablets) and dexamethasone (6 x 1.0 mg).
- Participant Score Sheets (see Appendix X)
- Checklist of items containing caffeine (see Appendix XXI)
- Watch, with second hand, to record timed tasks (i.e., FAS task, Spot the Word Task and Category Naming task).
- IBM Compatible Computer, to present: the Letters Item Recognition task and the Doors and Names recognition tasks.
- Glucose testing kit, comprising Softclix Pro and Lancets (Roche), Accutrend GC System (Boehringer Mannheim), BM Accutest test strips and instructions for use (see Appendix XI).
- Plasters and wipes.
- Vacutainers (3)

4.4.4. Serum cortisol levels

A qualified nurse took all blood samples at the end of each testing session. This meant that a total of three samples were taken from each participant on

three different days. Once obtained, the samples were then treated and analysed using the same procedure for serum cortisol described in Chapter 2.

4.4.5. Glucose Levels

A small sample of blood collected for the serum cortisol was pipetted from the vacutainer prior to spinning and used to measure glucose levels. The measurement and analysis of the samples was carried out using the same equipment and procedure for glucose samples described in Chapter 3.

4.4.6. IQ levels

A measure of IQ was obtained from each participant using the National Adult Reading Test (NART; Nelson & Willison, 1991). The NART comprises a list of fifty words that are printed in order of increasing difficulty. All of the words are irregular, in terms of the common rules for pronunciation, to reduce the possibility of the participant reading them correctly by phonemic decoding rather than word recognition. The words are also relatively short to avoid any possible adverse effects of 'stimulus complexity on the reading of dementing subjects' (Nelson & Willison, 1991). Full instructions for the administration and interpretation of the NART scores, together with a copy of the words used, are shown in Appendix XXIV.

The NART test was scored using the instructions specified for *Good readers*. This is where the NART error score is made up from the total number of errors made on the complete NART (i.e., maximum = 50). In terms of reliability and validity, the reliability of the NART assessed by the Cronbach alpha technique has shown a reliability coefficient of 0.93. Studies

by Nelson and colleagues in 1975 and 1978, comparing the NART scores produced by patients with dementia compared with those produced by normal controls, have also shown the NART to be a valid and useful technique for estimating premorbid IQ levels in dementia (Nelson & McKenna, 1975; Nelson & O'Connell, 1978). More significantly, however, in terms of this current study, the NART has 'obvious potential as a criterion for group matching in research studies', as well as being a quick test to administer which can also be pleasant for the participant.

4.4.7. Other measures

The following additional measures were also obtained from each participant during the first testing session:

- Age.
- Height and weight, to calculate BMI.
- Number of years diagnosed with Addison's disease and current medication regime for Addisons.
- Details of any other current illnesses and medication details.
- Current depression levels (i.e., BDI-21 scores). These were obtained using the same procedure as described in *Beck Depression Inventory, Version 21 (Beck et al., 1961)* in Chapter 3.
- Current anxiety levels (i.e., GHQ-30 scores). These were obtained using the same procedure described in *General Health Questionnaire, Version 30 (Goldberg, 1972; 1978; Goldberg & Williams, 1988)* in Chapter 3.

The following additional measures were also obtained from each participant at each testing session:

- Approximate caffeine intake from 24 hours prior to testing. This was obtained for the same reasons and using the same checklist described under *3.4.6. Other measures* in Chapter 3.
- List of food items consumed during the day prior to testing. This was also obtained for the same reasons described in *3.4.6.Other measures* in Chapter 3.

The information provided from the above measures was then analysed to see if there were any relationships with memory performance.

In addition, each participant was asked to complete Version A of the Spot the Word test, which forms part of the Speed and Capacity of Language Processing task (Baddeley et al., 1992). This task was carried out as a secondary measure of IQ and was only administered once to participants under the MRs/GRs condition; this was to ensure that all participants were tested under the same condition. It was also carried out during the MRs/GRs condition as, under normal conditions, both the MRs and GRs are activated by cortisol levels.

The Spot the Word task was carried out using the same procedure described in *Speed and Capacity of Language Processing (Baddeley et al., 1992)* in Chapter 3. As this test has also been described as a test of IQ, the data produced was compared to that produced using the NART; this was to see if there was any relationship between the two. However, the results of a

Spearman's rho correlation (used because the data were not normally distributed) showed no significant relationship (i.e., $r_s = 0.378$; NS). Indeed, the Rsq value showed that IQ score only explained approximately 28% of the variance in Spot the Word scores.

4.4.8. Medication

The doses and types of steroids used to activate the different corticosteroid receptors for the three testing conditions are shown in Table XXII. Fludrocortisone was used to activate the MRs only; dexamethasone was used to activate the GRs only; and a combination of both fludrocortisone and dexamethasone was used to activate both receptor-types. The doses of medication were determined by Professor Lightman.

To prevent any expectation of effects, participants were not made aware of the condition they had been allocated to or what the medication was they had been given to take until the debriefing session at the end of the second testing session. For safety reasons, however, the investigator knew which condition each participant had been allocated to so she could be contacted, using a 24-hour contact number which was given to each participant, in case of an emergency (e.g., adverse reactions).

All medication was prepared and pre-packed by the hospital pharmacy in the Bristol Royal Infirmary and supplied for self-administration in tablet form. The tablets were supplied in bottles which had been labeled with either Condition 1, 2, 3(a) or 3(b) as shown in Table XXII. In addition to the instructions provided by the researcher, instructions for administering the tablets were also given on each label.

Table XXII : Showing steroids and doses used to activate different corticosteroid receptors.

Medication	Time of Day of administration	Total Dosage (no of tabs)	Additional instructions
Fludrocortisone to activate MRs only Label – Cond 1	Between 07.00 - 08.00 hrs	0.2 mg (2 x 0.1 mg tabs)	Participants were instructed to: • Cease taking normal medication after evening of three days prior to testing. • Take a total of TWO tablets only per day, on each of the three mornings leading up to testing (i.e., participants were given a total of SIX tablets). There were NO tablets to take in the afternoon. • To recommence normal treatment regime on afternoon of testing day.
Dexamethasone to activate GRs only Label – Cond 2	Between 07.00 - 08.00 hrs	1.0 mg (2 x 0.5 mg tabs)	Same as above
Fludrocortisone – Label – Cond 3(a) **Dexamethasone** - Label – Cond 3(b), to activate both receptor types	Between 07.00 - 08.00 hrs	2 x 0.1 mg tabs AND 2 x 0.5 mg tabs	Same as above, but to take a total of TWO of each tablet each day, making a total of FOUR tablets each day.

4.4.9. Memory Tests

The effects of the different types of steroids on memory performance were tested using three different, but counterbalanced, batteries of memory tests. Each battery comprised three versions of eight different memory tasks, which were each designed to take no longer than 45 minutes to complete. The tasks used, order and method of delivery were the same as those detailed in Table X in Chapter 3, with the exception of the Spot the Word Task. As described above, only Version A of the Spot the Word task was used and this was given to participants as part of the MRs/GRs testing session only. The tasks were

always presented in the same order, using exactly the same procedures as described in Chapter 3.

- *Allocation of tasks to each battery*

Each participant completed three different batteries of memory tasks. Each battery comprised one of three different but comparable versions of nine memory tasks. The tasks were randomly allocated to each battery by the researcher by withdrawing one of three tickets from each one of seven bags (i.e., each memory task had it's own bag which contained three labels detailing each of the different task versions). The tasks used were the same as those described in Chapter 3, plus one additional version of each task.

4.4.10. Procedure

- *Recruitment*

Recruitment into the study started in January 2000 and took place over four phases. All correspondence sent out as part of the recruitment process had received full ethical approval from the United Bristol Healthcare NHS Trust.

- *Phase I – Letter Phase.* A total of 50 patients were initially invited to take part in the study by letter. The names and addresses of these patients were obtained using details held on a database at the Bristol Royal Infirmary (see Appendix XVI for a copy of the letter which was sent out). The letters gave a brief outline of the study, together with a response slip that the patients were asked to complete and return using a self-addressed envelope (SAE) if they were interested in taking part. If interested, they

were also asked to indicate, using the same response slip, whether they would be happy to receive further information as a group, or prefer to receive it independently. The letters were sent out from the Department of Medicine and signed by Professor Lightman. Out of the total of 50 patients who were sent letters, 16 expressed an interest in taking part, 9 replied saying they were not interested and 25 did not reply. In addition, all interested patients replied saying that they were happy to receive further information as a group.

- *Phase II – Meeting.* All participants who showed an interest in the study were contacted and invited to attend a short group meeting to hear more about the study and ask any questions; they were also informed that this meeting would last approximately one hour. This meeting was held at the Clinical Investigation Unit in the BRI and was hosted by Professor Stafford Lightman and the researcher. At the end of the meeting, each patient was then given two further sheets for completion. The first sheet asked the patient if s/he was still interested in taking part in the study and if so, to provide further information, including their GP's name and address. The second sheet was a consent form (see Appendices III and XIX for copies of these sheets). Both sheets were given out to patients with an SAE, with instructions for them to take them home and complete and return them at their earliest convenience. The reason for asking patients to complete the forms at home was to give them some extra time think about their decision and to save any embarrassment for those who did not wish to take part.

All patients who showed further interest in taking part in the study after the initial information meeting did so by returning their information sheet and consent forms, duly completed. Out of the 16 patients who attended the meeting, 10 patients said they would like to take part. As part of the response form, all interested patients were also asked to provide details of which days during the week would be most convenient for them to attend testing sessions. This included weekends.

- *Phase III : GP Contact.* By using the details provided on the response sheet, each participant's GP was sent details of the study and informed that their patient was interested in taking part. This was to give GP's the opportunity to make any comments if they felt that their patient should not take part (see Appendix XIX for details of the letter sent out to GP's). No comments to this effect were made by any of the GP's.

- *Phase IV – Dates for testing.* Also by using the details provided on the response sheet, the researcher contacted each patient by telephone to arrange a date for the first testing session.

- *Phase V - Confirmation and instructions letter.* The first date for testing was confirmed by letter. This information was sent by registered post as it also contained the tablets and instructions for each testing day (see Appendix XXI for a copy of the full instructions). The information sheet provided the following details:

- The actual date that the patient would need to discontinue their normal Addison's medication regime (on the 3rd evening prior to the testing day).
- The actual date that the patient would need to start taking the 'testing' medication (on the morning after they had discontinued their normal medication).
- The number of tablets they would have to take on each of the three mornings prior to testing (between 07.00 hrs and 08.00 hrs).
- Instructions on what to do from 24 hours prior to testing (to remain alcohol- and/or recreational drug-free and record approximate caffeine-intake using the enclosed checklist).
- Instructions on what to do on the day of testing before arriving at the CIU for 10.45 hrs (to have taken final tablets by 08.00 hrs and not to eat anything after 09.00 hrs).
- Instructions on what to do upon arrival at the CIU (to report what they had eaten for breakfast; report how stressed they felt; complete a battery of memory tasks at 11.00 hrs; and provide an intravenous blood sample to measure steroid and glucose levels).
- Instructions on what to do after testing (to make arrangements and obtain tablets for next testing session, and resume normal Addison's medication regime that afternoon).

- *Phases VI, VII and VIII - Induction phase and testing sessions.* The format of the first testing session was the same as for testing sessions 2 and 3, but with the addition of the induction phase:

- *Induction phase*

  The induction phase was carried out during the first part of testing session 1. During this phase, each participant was asked to provide information relating to the measures described in *4.4.7. Other measures*. This was apart from completing the Spot the Word task, which was administered during the MRs/GRs condition only.

- *Testing sessions 1, 2 and 3.*

  All memory testing was carried out at 11.00 hrs in the CIU in the BRI.

  As part of the testing session, all participants were asked to:

  - Follow the instructions sent out with the tablets.
  - Arrive at the CIU for 10.45 hrs with completed caffeine checklist.
  - Report what they had eaten for breakfast.
  - Report how stressed they felt on a scale from 0 to 10.
  - Complete a battery of memory tests at 11.00 hrs.
  - At the end of the testing session, to provide intravenous plasma cortisol and glucose samples to a qualified nurse.
  - Make arrangements for next testing session and collect instructions, tablets and caffeine checklist.

A flow diagram showing the design of the study and a summary of the procedure for each condition is shown in Figure 14.

Figure 14 : Flow diagram summarising the design of the study

Stage I

Recruitment carried out by letter →

This included a response slip which patients had to complete and return (using SAE) if they wanted to discuss the study further. They were also asked to specify if they would /would not be happy to discuss the study as a group.

↓

Stage II

Letter sent out to interested patients with date of meeting at BRI. →

The meeting was held at the BRI, during which patients were given:

- further information about the study;
- a chance to ask questions of Prof. Lightman and the researcher;
- a second letter, which included a response sheet and consent form for the patient to complete and return (using SAE) if they wished to become a volunteer in the study . This also asked patients to give most suitable days for testing.

↓

Stage III

Letter sent to patient's GP

Letter sent to volunteers with selection of dates for testing →

This provided details about the study and informed GP that one of their patients was taking part.
Based on the information provided by the patient after Stage I, this asked volunteers to tick dates available for testing (which had previously been agreed with the nurse) and return using SAE. The researcher used this information to arrange the first testing session.

↓

Stage IV

Volunteer contacted by telephone to arrange first testing session →

The researcher made this contact.

↓

Stage V

Registered letter sent to volunteers to confirm details of first testing session. This included tablets and instructions for each testing day →

This informed the participant that they would have to follow the instructions detailed in Appendix XXI.

↓

Stage VI

First testing session/induction meeting →

During this session, participants were asked to:

- Get up at 08.00 hrs and start taking medication (as per instructions)
- Not to eat anything from two hours prior to testing
- Arrive at CIU for 10.45 hrs

			• Report perceived levels of stress using likert scale from 0 – 10. • Report any side effects. • Give details of any serious family history of illness, year of diagnosis of Addison's and current treatment regime* • Report details of any other current illness and medication* • Provide age, height & weight details* • State if HIGH, MODERATE or LOW caffeine user* • Complete NART, BDI and GHQ questionnaires* • Produce checklist of items containing caffeine consumed from 24 hours prior to testing • Provide details of all food items eaten on day prior to testing • Complete first battery of memory tests • Provide blood samples to measure glucose and levels of serum cortisol • Arrange dates for next testing session • Take tablets and instructions for next testing session.
	↓		
Stage VII	**Second testing session**	→	Same as for Stage V, excluding items marked with *
	↓		
Stage VIII	**Third testing session**	→	Same as for Stage VI, except for arranging dates for next testing session etc. Participant was also debriefed and given opportunity to ask any questions

At the end of the final testing session, all participants were debriefed and thanked for their participation in the study. They were also given an opportunity to ask questions.

- *Phase IX – Debriefing information and summary of results*

  Once all the testing sessions had been completed, a comprehensive written version of the debriefing information was sent out to each participant, together with a summary of their memory testing results. (See Appendix XXV for details of debriefing information and copy of summarised results.)

4.5 Results

The purpose of this study was to examine the effects of different acute changes in steroids (which were administered to activate the different corticosteroid receptors) on working memory and the episodic and semantic components of declarative memory. Consequently, the most important data reported in this results section are the actual levels of memory performance produced under each of the three conditions. However, as for Chapter 3, the first part of this results section will focus on the other measures obtained during the study which previous researchers have shown can modify the effects of cortisol on memory performance. These include the participants' characteristics, levels of caffeine and items of food consumed prior to testing, and glucose levels. The reason for doing this is to identify which of these variables did appear to have an influence on memory performance.

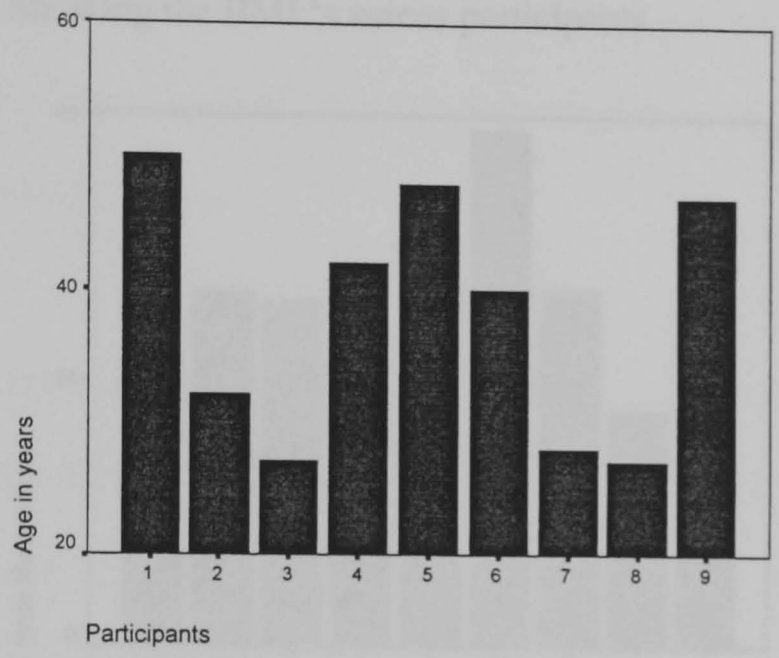
4.5.1. Participants' Characteristics

As described earlier, a total of eleven participants were initially recruited into the study. However, two participants failed to complete all three testing sessions due to ill health during the MRs only condition and, consequently, the data presented in this results section are for a total of nine participants only. These comprised five males and four females. Full details of the mean ages, BMI's, depression levels, anxiety levels and IQ scores by sex are shown in Table XXI. The distributions of these measures across the nine participants are also shown in Figures 15-19.

- *Age and memory performance*

The mean age of participants was 37.9 years (SD = 9.51). Figure 15 shows the actual ages of participants.

Figure 15 : Showing the ages of participants

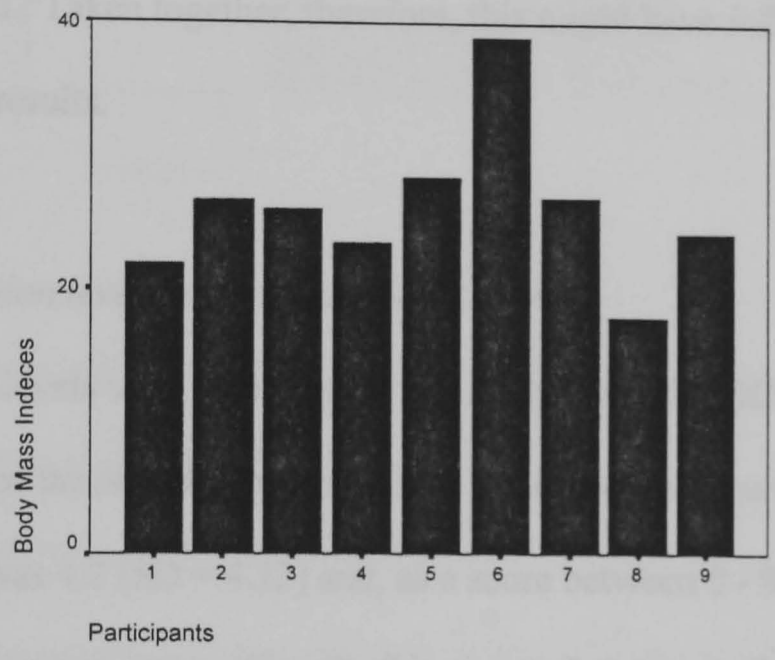


A series of Spearman's rank correlations were carried out (because the data were not normally distributed) to see if there were any relationships between age and any of the different aspects of memory performance; memory performance was measured irrespective of condition using the total scores obtained for each task type. These results showed that, apart from the significant and positive relationship between age and total scores for the category naming task (rho = 0.695; $p<0.05$), which suggests that an individual's ability to name categories is enhanced with age, there were no significant relationships between age and any of the other aspects of memory performance. (See Appendix XXVII for full details of the results.)

- *Body mass index and memory performance*

The BMI index of each participant was calculated using the same formula described in Chapter 3. The BMI's for participants in this study is shown in Figure 16.

Figure 16 : Showing the BMI 's across participants



The mean BMI score was 26 (SD = 5.79). The range of BMI scores considered to be within the normal healthy range is between 20 and 24, with anything < 20 considered to be underweight and anything > 24 considered to be over-weight. In line with this interpretation, Figure 16 shows that whilst one participant would be considered underweight and there were three participants within the normal healthy range, the majority of participants in this study would be considered over-weight. As weight gain is a common side-effect of steroids, this observation was not too surprising.

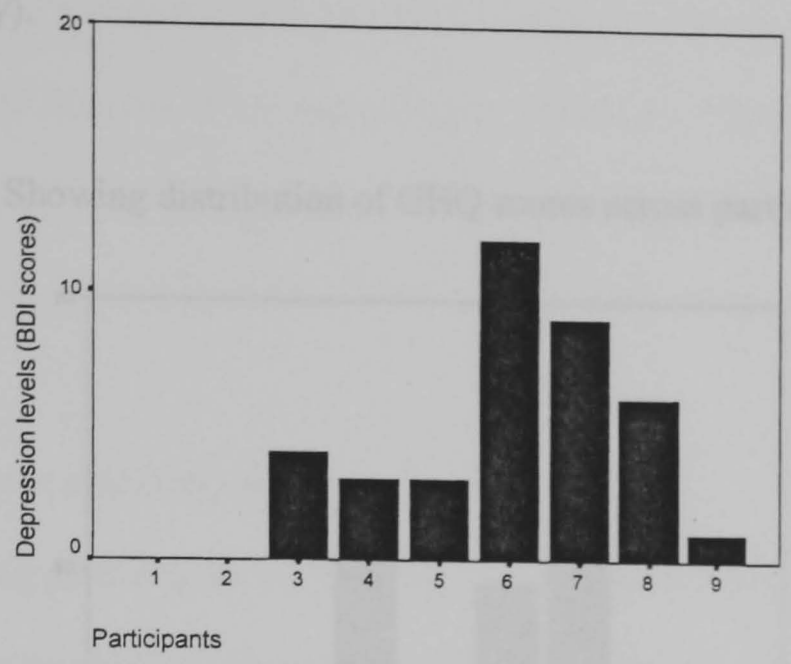
A series of Pearson's Product Moment correlations (because the data were normally distributed) were carried out to see if there were any relationships between BMI and memory performance, irrespective of condition. However, apart from a significant and positive correlation between

BMI and total HVLT recognition scores ($r = 0.697$; $p < 0.05$), which suggests that recognition performance on this task was enhanced by body size, there were no significant relationships between BMI and any of the aspects of memory performance. (See Appendix XXVII for full details of the results.) It is important to note that, as for Experimental 2, the scores produced by participants for the HVLT recognition task in this study were nearly all at ceiling level. Taken together, therefore, this might have influenced these significant results.

- *Depression levels and memory performance*

Depression levels were measured in this study using the BDI questionnaire and details of the actual scores produced are shown in Figure 17. The mean BDI score was 4.2 (SD = 4.12) and, as a score between 0 - 9 is considered to be with the normal range of levels, this shows that one participant (who was female) experienced mild levels of depression during 'the past week, including today' when completing the questionnaire. It also shows that two participants (also female) did not report having felt depressed during the week prior to testing.

Figure 17 : Showing BDI scores across participants



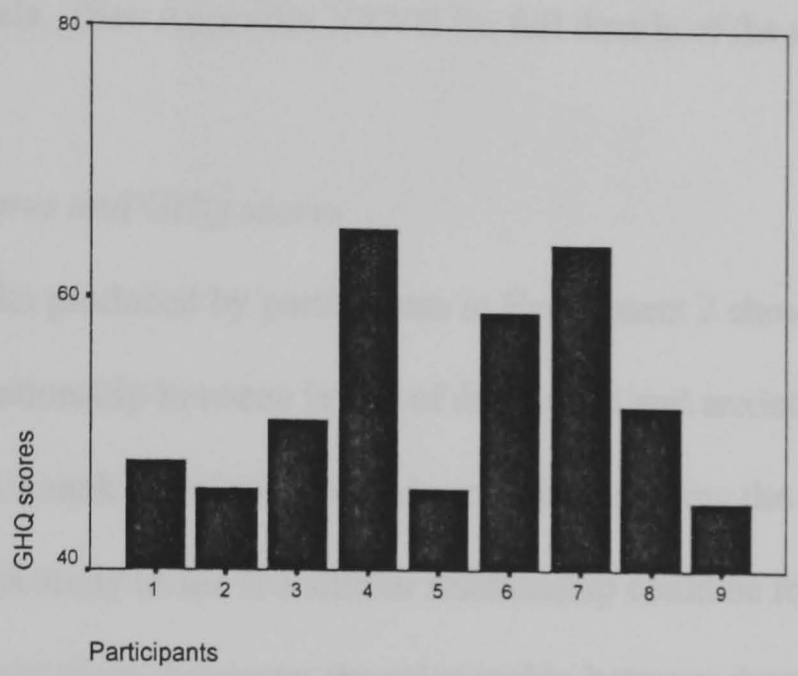
A series of Spearman's rank correlations were carried out to see if there were any relationships between BDI scores and any of the aspects of memory performance irrespective of condition. However, apart from a significant and negative relationship between BDI scores and total digits forward scores (rho = -0.835; $p < 0.01$), which suggests performance on the digits forward task was impaired by depression levels, the results showed no significant relationship between BDI scores and any of the other aspects of memory performance. (See Appendix XXVII for full details of the results.)

- *Anxiety levels and memory performance*

Anxiety levels were measured using the GHQ and the scores produced are shown in Figure 18. The mean GHQ score was 52.9 (SD = 7.85) and, as a score > 60 indicates higher levels of anxiety, Figure 19 shows that most of the participants in this current study produced scores within the normal anxiety level range. Participants 4 and 7, however, showed higher levels of anxiety

when completing this questionnaire (i.e., they produced scores of 65 and 64 respectively).

Figure 18 : Showing distribution of GHQ scores across participants



A series of Spearman's rank correlations were carried out to see if there was any relationship between anxiety levels and any of the aspects of memory performance irrespective of condition. In comparison to the other participant characteristics, these did show significant relationships between GHQ scores and several aspects of memory performance. More specifically, they showed significant and negative relationships between GHQ scores and the scores for three of the working memory tasks (i.e., with total digits backward scores [rho = -0.685; $p < 0.05$]; with total letter naming scores [rho = -0.689; $p < 0.05$]; and with total category naming scores [rho = -0.711; $p < 0.05$]). They showed significant and negative relationships between GHQ scores and two of the episodic memory tasks (i.e., with total HVLT recall scores [rho = -0.798; $p < 0.05$] and between total scores for the doors task [rho

= -0.678; p<0.05]). They also showed a significant and negative relationship between GHQ scores and with one of the semantic memory tasks (i.e., between total Spot the Word scores [rho = -0.844; p < 0.01]). In summary, therefore, these results suggest that memory performance can be impaired by anxiety levels. (See Appendix XXVII for full details of the results.)

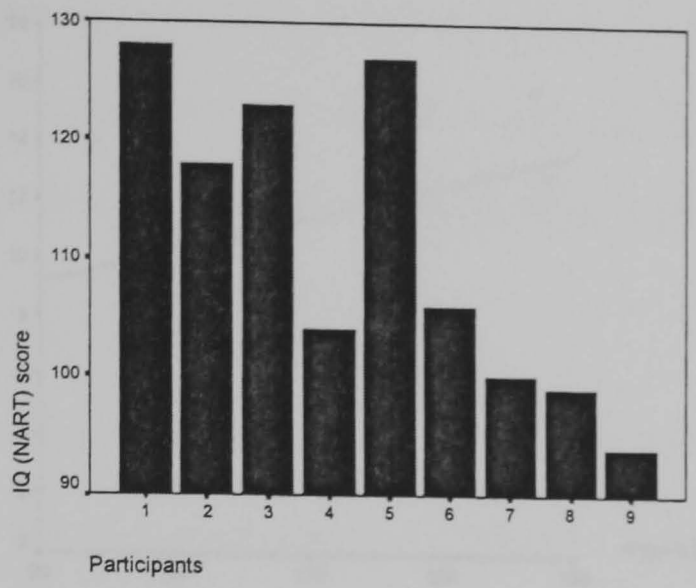
- *BDI scores and GHQ scores*

As the results produced by participants in Experiment 2 showed a significant positive relationship between levels of depression and anxiety (see Chapter 3), a Spearman's rank correlation was also carried out using the BDI and GHQ scores in this study to see if a similar relationship could be found in this study. Although very close, however, the relationship between depression and anxiety levels was not significant (r_s = 0.650; NS); this lack of relationship may be explained by the small sample size (N = 9).

- *Intelligence levels (IQ) obtained using NART scores and memory performance*

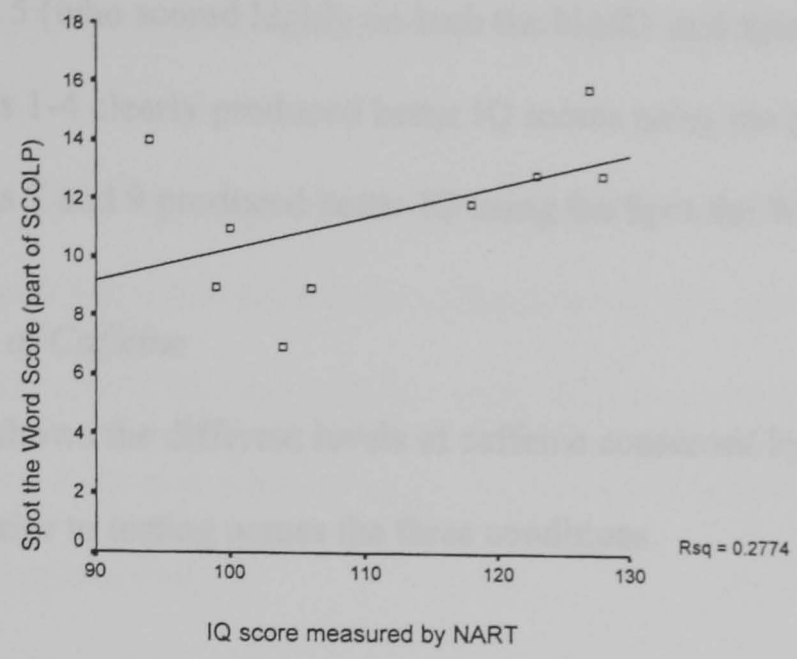
The NART was used to obtain a measure of intelligence levels for each participant. The distribution of scores produced are shown in Figure 19. As the mean IQ score was 111 (SD = 13.07), the results showed that the majority of participants produced scores which were either equal to or higher than average (i.e., according to the Stanford Binet intelligence test, 90-109 is considered average). All the female participants also produced scores > 117 (i.e., they showed above average intelligence levels).

Figure 19 : Showing the distribution of IQ scores obtained by the NART



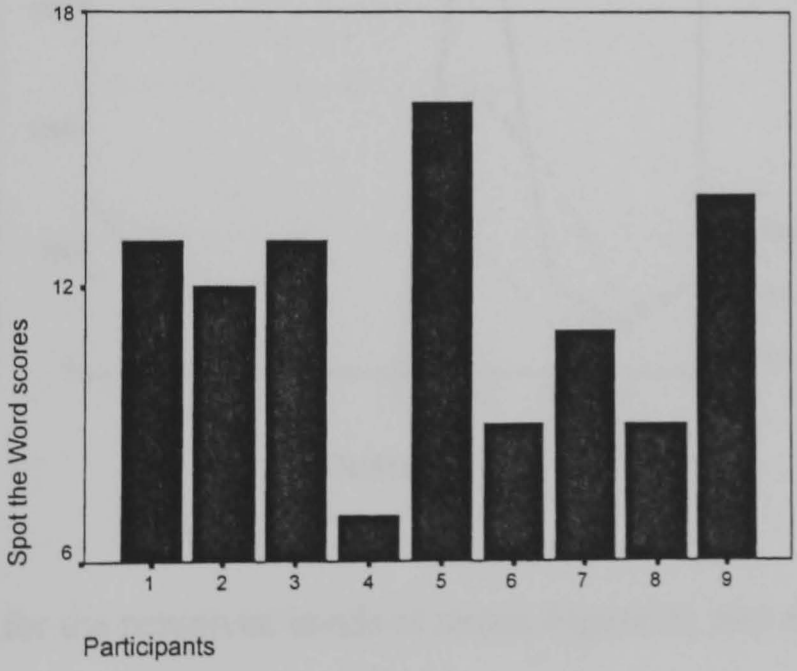
A series of Spearman's rank correlations were carried out to see if there was any relationship between IQ score and any of the aspects of memory performance irrespective of condition. The results showed no significant relationships between IQ scores and any of the aspects of memory performance; this was not predicted. (See Appendix XXVII for full details of the results.) What was also surprising was that the results of the Spearman's rank correlation also showed no relationship between the scores for NART and the scores for the Spot the Word task (rho = -0.378; NS). This is shown in Figure 20. As the scores produced by the Spot the Word task are also regarded as a measure of verbal fluency, this was not predicted (i.e., previous researchers reported a validity correlation of 0.831 for Version A of the Spot the Word task and the NART; Baddeley et al., 1992). A series of boxplots also showed no outlying scores to explain this lack of relationship.

Figure 20 : Showing relationship between NART and Spot the Word scores



The actual scores produced by participants for the Spot the Word task are shown in Figure 21.

Figure 21 : Showing Spot the Word scores across participants

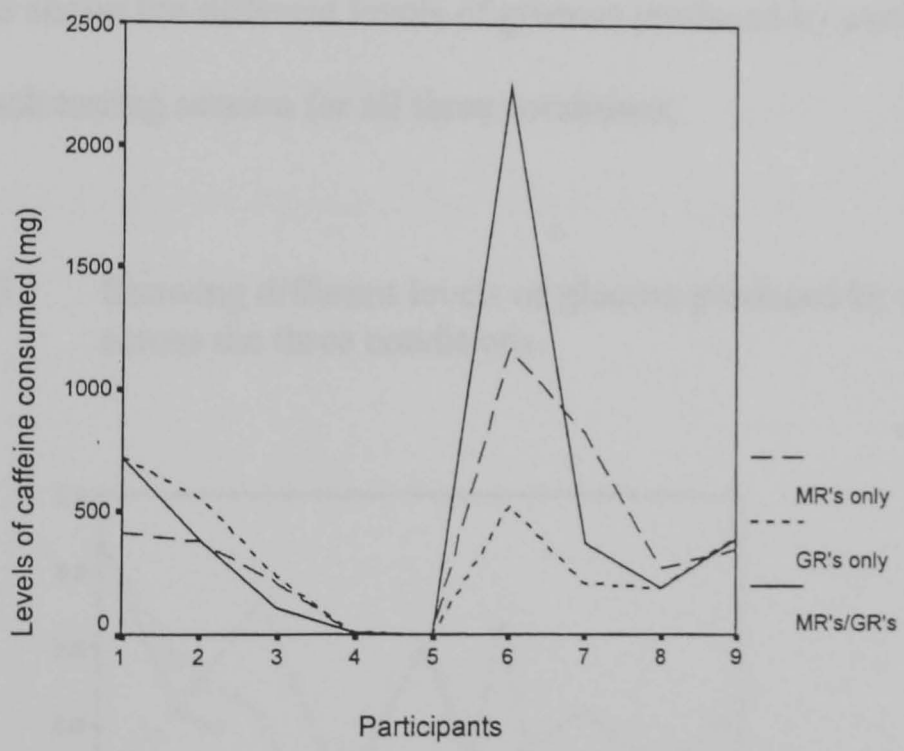


A comparison of this graph with Figure 20 shows that, apart from Participant 5 (who scored highly on both the NART and Spot the Word task) Participants 1-4 clearly produced better IQ scores using the NART, whereas Participants 7 and 9 produced better IQ using the Spot the Word task.

- *Effects of Caffeine*

Figure 22 shows the different levels of caffeine consumed by participants from 24 hours prior to testing across the three conditions.

Figure 22 : Showing different levels of caffeine consumed by participants across the three conditions.



As for the perceived levels of stress, Figure 22 also shows two sets of quite different results between participants 1 - 4 and participants 5 – 9 in the levels of caffeine consumed from 24 hours to testing across the three conditions. However, the results of a repeated measures ANOVA (which was carried out because the data met the assumptions for homogeneity of variance and sphericity) showed that the mean levels of caffeine consumed throughout

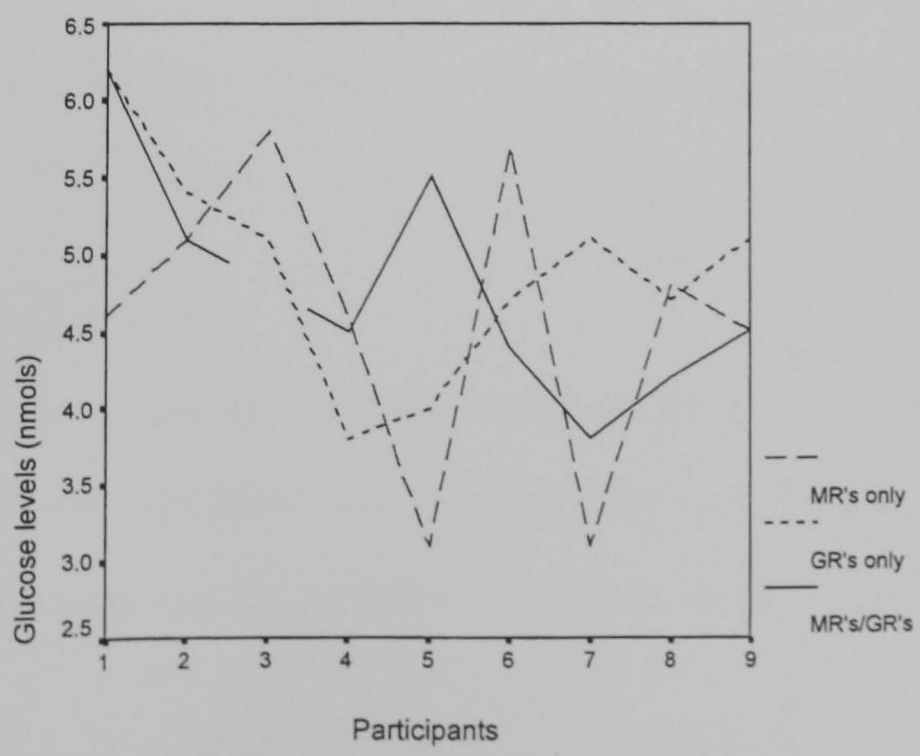
the day (i.e., mean = 401 vs. 315 vs. 490 mg for the MRs vs. GRs vs. MRs/GRs conditions respectively) were not significantly different ($F(2,16) = 0.657$; NS).

The effects of caffeine on the different aspects of memory performance were also investigated using a series of Spearman's rank correlations. However, the results showed no relationship between caffeine levels and any of the aspects of memory performance. (See Appendix XXVII for full details of the results produced.)

- *Effects of Glucose*

Figure 23 shows the different levels of glucose produced by participants at the end of each testing session for all three conditions.

Figure 23 : Showing different levels of glucose produced by participants across the three conditions.



The mean levels of glucose produced in each condition were 4.6 vs. 4.9 vs. 4.8 nmols for the MRs vs. GRs vs. MRs/GRs conditions respectively.

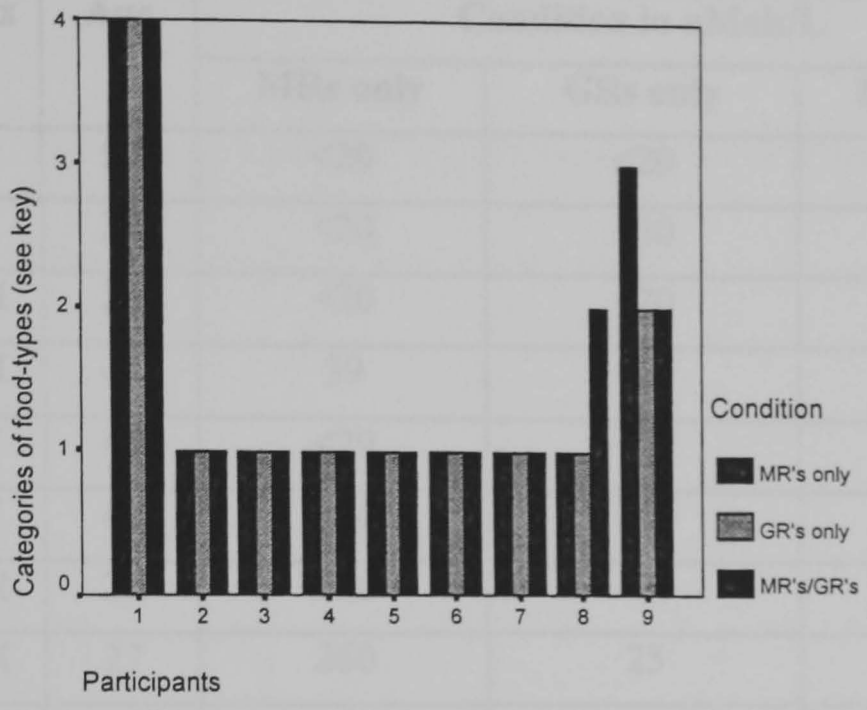
As shown, these levels were quite similar. Indeed, the results of a repeated measures ANOVA, which was carried out because the data met the assumptions for homogeneity of variance and sphericity, showed no significant differences in glucose levels ($F(2,14) = 0.766$; NS).

The effects of glucose on the different aspects of memory performance irrespective of condition were investigated using a series of Spearman's rank correlations. The results showed no relationships between glucose levels and any of the aspects of memory performance. (See Appendix XXVII for full details of the results produced.)

- *Effects of Food-type*

Figure 24 shows a breakdown of the types of food eaten by participants prior to each of the three testing conditions. This shows that most of the participants tended to eat the same types of foods prior to each testing session

Figure 24 : Showing the types of food consumed prior to testing under each condition



Key

1 = High carbohydrates; 2 = High proteins; 3 = High carbohydrates/High proteins; 4 = Fruit

Although the data were not normally distributed, the assumptions for homogeneity of variance and sphericity were met. Consequently, a repeated measures ANOVA was carried out on the food data. As predicted, the results showed that the differences in types of food consumed were not significant ($F(2,16) = 0.471$; NS).

4.5.2. Serum levels of cortisol

All levels of cortisol were obtained from blood samples at the end of each testing session. The approximate levels of serum cortisol produced by each participant under each one of the three conditions are shown in Table XXIII

Table XXIII : Showing serum cortisol levels produced by each participant in each condition

ID	Sex	Age	Condition in nMols/L		
			MRs only	GRs only	MRs/GRs
1	F	50	<20	<20	<20
2	F	32	<20	<20	<20
3	M	27	<20	<20	<20
4	M	42	39	<20	<20
5	F	48	<20	<20	<20
6	F	40	<20	<20	<20
7	M	28	<20	<20	<20
8	M	27	260	25	82
9	M	47	<20	<20	<20

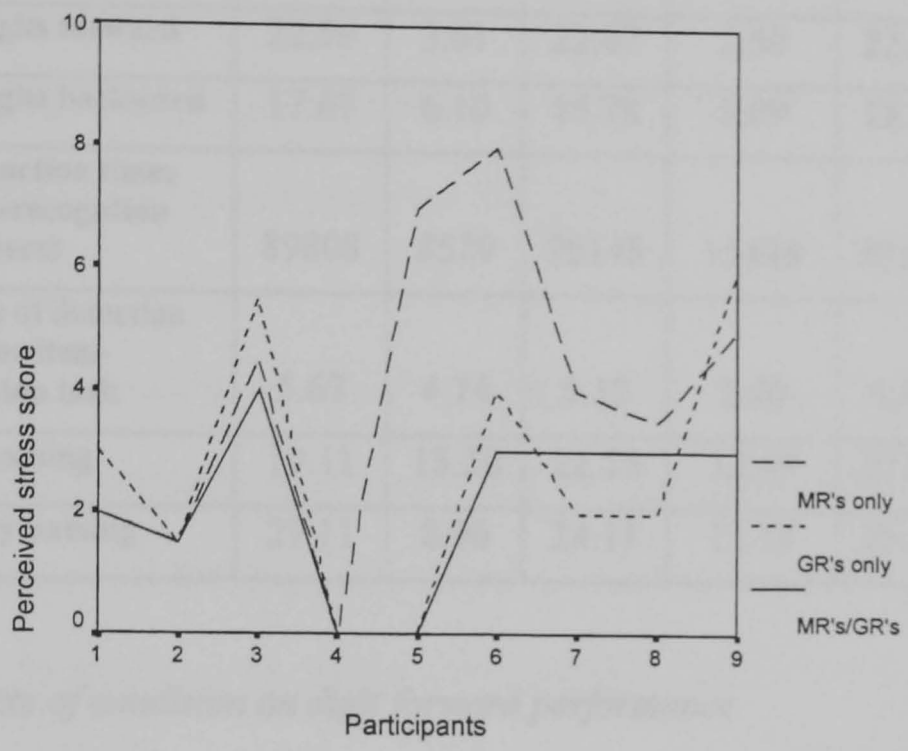
As shown, the levels of serum cortisol were very low, which was predicted as these participants have Addison's disease. The results also show that Participant 8 has a milder form of Addison's disease compared to the other participants. The levels shown do not exceed < 20 nMols/L due to the sensitivity of the immunoassays used to analyse the samples. Consequently, this meant that it was not possible to carry out any correlations to see if there was any relationship between serum cortisol levels and any of the aspects of memory performance.

4.5.3. *Perceived levels of stress*

All participants were asked to report their perceived levels of stress at the start of each testing session using the same Likert scale described in Chapter 3. The mean perceived stress levels for each condition were 3.9 vs. 2.7 vs. 2.2 for the

MRs vs. GRs vs. MRs/GRs conditions respectively and these are shown in Figure 25.

Figure 25 : Showing a comparison of perceived stress levels across conditions



This shows two completely different groups of results (i.e., those for participants 1 – 4 versus those produced by participants 5 - 9). As the data did not meet the assumptions for normality and homogeneity of variance required for a parametric test, a Friedman's test was carried out on the ranked data. This showed that the perceived levels of stress were not significantly different across the three conditions (Chi-square = 4.385; df = 2; NS). Consequently, these data suggest that activation of the different corticosteroid receptors using different steroids did not have any effects on perceived levels of stress.

4.5.4. Effects of condition on working memory performance

A summary of the mean scores and standard deviations for the individual working memory tasks completed under each of the three conditions is shown in Table XXIV.

Table XXIV : Mean scores and standard deviations for individual working memory tasks completed under each condition

Task	Condition					
	MRs only		GRs only		MRs/GRs	
	Mean	SD	Mean	SD	Mean	SD
Total digits forward	22.56	3.61	22.67	2.50	22.44	4.39
Total digits backward	17.67	6.10	15.78	5.09	18.56	6.41
Total reaction times for item-recogntion task (msecs)	89808	8529	95148	15449	90194	19043
Total no of detection errors for item-recognition task	5.67	4.74	5.12	2.90	3.00	1.66
Letter naming	19.11	13.20	22.56	12.49	21.56	12.26
Category naming	27.11	8.96	24.11	12.14	25.22	6.59

- *Effects of condition on digit forward performance*

The data for the digits forward tasks were not normally distributed and the assumptions for homogeneity of variance were not met. However, as the assumptions for sphericity were met a repeated measures ANOVA was carried out on the mean scores. The results showed that the differences in performance levels between participants were not significant ($F(2,16) = 0.038$; NS).

- *Effects of condition on digits backward performance*

The data for the digits backwards tasks were also not normally distributed. However, as the assumptions for homogeneity of variance and for sphericity were met a repeated measures ANOVA was carried out on the mean scores. The results showed that the differences in performance levels between participants were significant ($F(2,16) = 4.958$; $p < 0.05$). A series of post-hoc

pairwise comparisons were also carried out between the different conditions. This showed a significant difference in performance levels when participants received GRs only compared to MRs/GRs (i.e., for mean scores of 1.70 vs. 2.44, $t = -3.571$; $df = 8$; $p < 0.01$). No other significant pairwise comparisons were found. It can be concluded, therefore, that participants who received GRs only made significantly more errors on the digits backwards task than those who received a combination of MRs/GRs.

- *Effects of condition on reaction time during item-recognition performance*

The data for the total reaction time during the item-recognition tasks were not normally distributed and the assumptions for homogeneity of variance were not met. However, as the assumptions for sphericity were met a repeated measures ANOVA was carried out on the mean reaction times. The results showed that the differences in performance levels between participants were not significant ($F(2,16) = 0.469$; NS).

- *Effects of condition on number of detection errors made during item-recognition performance*

The data for the total number of detection errors made during the item-recognition tasks were not normally distributed, and the assumptions for homogeneity and sphericity were not met. Consequently, the data were transformed using a logarithmic transformation to enable a parametric repeated measures ANOVA to be carried out. The results showed that the differences in performance levels between participants were not significant ($F(2,14) = 1.214$; NS).

- *Effects of condition on letter naming performance*

The data for the letter naming tasks were also not normally distributed. However, as the assumptions for homogeneity of variance and for sphericity were met a repeated measures ANOVA was carried out on the data. The results showed that the differences in performance levels between participants were not significant ($F(2,16) = 3.657$; NS). It is also important to note, however, that participant 5 produced extremely high scores in the letter naming task in all three conditions. However, even when these scores were removed, the results remained non-significant ($F(2,14) = 2.549$; NS).

- *Effects of condition on category naming performance*

The data for the category naming task were not normally distributed and the assumptions for homogeneity of variance were not met. However, as the assumptions for sphericity were met a repeated measures ANOVA was carried out on the data. The results showed no significant differences in performance levels between conditions ($F(2,16) = 0.284$; NS).

- *Summary of effects of condition on working memory performance*

In summary, therefore, the results of this study found that participants produced the lowest digits backwards scores during the GRs only condition; this was predicted. The results also showed that participants produced the highest digits backwards scores when both receptors were activated. This suggests that participants show better performance levels when both receptors are activated as opposed to when only one receptor is activated, as predicted.

4.5.5. *Effects of condition on episodic memory performance*

A summary of the mean scores and standard deviations for the individual declarative memory tasks completed under each of the three conditions is shown in Table XXV.

Table XXV : Mean scores and standard deviations for individual declarative memory tasks completed under each condition

Task	**Condition**					
	MRs only		**GRs only**		**MRs/GRs**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Hopkins recall	26.22	6.60	28.00	3.61	31.89	2.89
Hopkins recognition	11.78	0.44	12.00	0.00	11.56	0.53
Names	35.22	2.28	36.00	2.40	35.11	2.20
Doors	32.78	4.55	33.89	3.69	32.22	4.38

- *Effects of condition on HVLT recall performance*

The data for the HVLT recall task were not normally distributed and the assumptions for homogeneity of variance were not met. In addition, participant 7 produced an extreme score of 25 under the MRs/GRs condition. Consequently, the data were transformed using a logarithmic transformation to enable a parametric test to be carried out. A repeated measures ANOVA was carried out on the transformed data and this showed significant effects of condition ($F(2,16) = 6.143$; $p=0.01$). Moreover, the results of a post-hoc analysis using a series of pairwise comparisons showed significant differences in recall scores between the GRs only and MRs/GRs condition ($p<0.05$) and between the MRs only and MRs/GRs condition ($p<0.01$) only. As for digits

backwards performance, this suggests that the detrimental effects of corticosteroids on recall performance are greater when only one corticosteroid receptor is activated in comparison to when both receptors are activated. The results also showed that recall performance was impaired to a greater extent by activation of the MRs only in comparison to activation of the GRs only. This effect is in the opposite direction to that identified in working memory. Consequently, this suggests that the detrimental effects brought about via activation of one corticosteroid receptors only as opposed to two may depend on which aspect of memory is being tested (i.e., working memory vs. episodic memory). It also supports the similar effect identified by Oitzl & De Kloet (1992) in rats.

- *Effects of condition on HVLT recognition performance*

As shown in Table XXV, the mean scores for the HVLT recognition tasks in each condition were generally at ceiling level (i.e., nearly all participants scored the maximum score of 12). Indeed, in the GRs only condition, all participants produced a score of 12. The data were not normally distributed and the assumptions for sphericity were not met. However, the assumptions for homogeneity of variance were met. Consequently, a repeated measures ANOVA was carried out on the HVLT recognition scores and, as suggested by the ceiling scores, the results showed no significant difference in recognition performance between the three conditions (i.e., $F = 2.286$; $df = 1.225$; NS).

- *Effects of condition on performance on the Names task*

In addition to the HVLT recall task, the Names and Doors tasks were also used to measure the effects of different steroids on recall performance. The data for the Names task were not normally distributed. However, as there were no extreme scores and the assumptions for homogeneity of variance and sphericity were met, a repeated measures ANOVA was carried out. In contrast to the HVLT recall task, however, the results of this analysis showed no significant effects of condition on recall performance ($F(2,16)$; 1.345: NS).

- *Effects of condition on performance on the Doors task*

The data for the Doors task were also not normally distributed and, although the assumptions of homogeneity of variance and sphericity were met, there were outlying scores. Consequently, the data were transformed using a logarithmic transformation to enable a repeated measures ANOVA to be carried out. The results of this also showed no significant difference between the three conditions in recall performance ($F(2,16) = 0.690$; NS).

- *Summary of the effects of condition on episodic memory performance*

In summary, therefore, the results of this study showed that, in contrast to the effects on working memory, participants generally produced the lowest episodic memory scores (using the HVLT recall task) following activation of the MRs only compared to GRs only. The results also showed that, as for working memory performance, as predicted participants showed higher levels of HVLT recall performance when both receptors were activated as opposed to when only one receptor type was activated.

4.5.6. *Effects of condition on semantic memory*

In addition to the episodic component of declarative memory, the effects brought about via activation of the different corticosteroid receptors on the semantic aspect of declarative memory were also investigated. This was done using the Speed of Processing task. A comparison of the mean scores produced for each task and for each condition is shown in Table XXVI.

Table XXVI : Showing mean scores produced by semantic memory tasks for each condition.

Task	**Condition**					
	MRs only		**GRs only**		**MRs/GRs**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Speed of Processing	13.89	3.22	14.33	3.28	14.44	3.32

- *Effects of condition on Speed of Processing performance*

The scores for the speed of processing task were not normally distributed. However, as the assumptions for homogeneity of variance and sphericity were met a repeated measures ANOVA was carried out on the data. This showed no significant difference between the conditions ($F(2,16) = 0.471$; NS).

In summary, therefore, the results of this study showed that the effects of corticosteroids on semantic memory performance were not affected by activation of the MRs only. However, as for the other two aspects of memory performance, they did show that participants performed better when both receptors are activated; this was predicted.

4.5.7. *Relationship between duration of treatment with steroids and memory performance*

As mentioned previously, in a longitudinal study Keenan et al. (1995) identified a significant age by duration effect on declarative memory performance during the first three years of long-term treatment with steroids. However, this was only apparent in the older participants, aged > 45 years. The mean duration of treatment with steroids received by participants in this study was 11.33 years, with individual durations of treatment ranging from 5 – 27 years. Consequently, as Keenan et al. only identified a significant age by duration effect during the first three years, it was doubtful that any effect would be found in this current study.

However, whilst the results of a series of Spearman's rank correlations between duration of treatment with steroids and memory performance irrespective of condition and age showed no significant relationships between the two (see Appendix XXVII for results), the results produced by the participants who were > 45 years (N=3) showed several significant relationships which the participants who were < 45 years (N=6) did not. More specifically, the results produced by participants > 45 years showed significant relationships between duration of treatment and performance levels (irrespective of condition) for the: total digits forward task (rho = -1.000; $p < 0.001$); total number of detection errors made during the item-recognition task (rho –1.000; $p < 0.001$); and the doors task (rho = 1.000; $p < 0.001$). It was also interesting that the relationships between duration of treatment and working memory performance were negative; these suggested detrimental effects on digits forward performance but beneficial effects on item-

recognition performance. The relationship between duration of treatment and performance on the episodic memory task, however, was positive: this suggested detrimental effects on performance. Whilst the detrimental effects might have been predicted, the beneficial effects of long-term treatment with steroids would not (although the beneficial effects may actually be showing a plateauing effect). In summary, therefore, the results of this part of the study suggest that there might be a relationship between duration of treatment with steroids and memory performance, with other opposing effects.

4.6. Discussion

The overall aim of the current study was to investigate whether acute changes in corticosteroid receptor activation in a population of participants who had received chronic levels of treatment with steroids had any effects on memory performance. More specifically, to see whether balanced activation of both the MRs and GRs is necessary for optimal memory function. To do this, therefore, the effects on working memory and the episodic and semantic components of declarative memory were examined under each one of three different conditions. These comprised: following activation of the MRs only, using fludrocortisone; following activation of the GRs only, using dexamethasone; and following activation of both receptor types using fludrocortisone and dexamethasone. The results of the study showed significant detrimental effects on working memory performance (using the digits backward task) following activation of the GRs only (i.e., $p < 0.05$). They also showed significant detrimental effects on episodic memory (using the HVLT recall task) following activation of the MRs only (i.e., $p = 0.01$). Consequently, whilst these results were not consistent across all of the memory tasks, as previously identified in rats (Oitzl & De Kloet, 1992) they do go some way to suggest that activation of the MRs only affects declarative memory performance and activation of the GRs only affects working memory performance in humans. The results also showed that individuals performed better in all aspects of memory following activation of both receptors (i.e., MRs and GRs). This supports the claim that, in contrast to activation of MRs only or GRs only, optimal memory function depends on balanced activation of both the MRs and GRs (De Kloet et al., 1999).

A potential explanation for why all of the working memory and episodic memory tasks did not identify significant detrimental effects on memory may be

related to methodology. Specifically that, although the tasks used are reliable and valid, they may not have been sensitive enough to detect these effects. As described previously, prior to this study the only other previous research to have looked at the effects on memory following differential activation of the MRs versus GRs was carried out in rodents (e.g., Oitzl & De Kloet, 1992; Sandi & Rose, 1994). The effects were also identified using tasks like the spatial navigation Morris water maze task; this task is not ethologically relevant to humans. Consequently, there are no other studies that can support the reliability and validity of the tasks used in this study for examining the effects produced by differential activation of the receptors.

4.6.1. Effects of chronic duration of treatment with steroids on memory

In addition to the effects on memory performance produced following activation of the different corticosteroid receptors, a second aim of this study was to see if there were any effects of duration of treatment with steroids on memory performance per se. Although activation of the different receptors was acute in terms of treatment duration (i.e., for 48 hours only), as patients with Addison's disease are treated life-long with replacement levels of cortisol using steroids, this study provided a further opportunity to explore this. To do this, therefore, a series of Spearman's rank correlations were carried out on the data. However, the results showed no significant relationships between duration of treatment with any of the different aspects of memory performance.

The lack of any baseline measures of memory performance for patients before they began treatment with steroids is one of the limitations of this study. Moreover without these, this makes the identification of a true

relationship between duration of treatment and effects on memory performance impossible to find. However, the results of this study did identify several significant relationships between age and duration of treatment (irrespective of condition) in the older aged participants (i.e., those who were aged older than 45 years; N = 3). More specifically, the results showed significant relationships between duration of treatment and working memory performance in total digits forward performance (rho = -1.000; $p < 0.001$), and in total number of detection errors made during the item-recognition task (rho = -1.000; $p < 0.001$). They also showed significant relationships between duration of treatment and episodic memory performance using the doors task (rho = 1.000; $p < 0.001$). There were, however, no significant relationships between duration of treatment and memory performance identified by any of the other tasks, or by these same tasks in the younger aged participants (i.e., those who were aged younger than 45 years; N = 6). As described in Chapter 1, Keenan et al. (1996) identified greater memory deficits with less protracted treatment with prednisone in patients aged > 45 years compared to younger patients. The elderly are also at greater risk for increased cognitive impairment and show a higher cortisol response to stress (Meaney et al., 1995) Consequently, the results of this current study also suggest that the detrimental effects of steroids on memory performance may increase with age.

Keenan et al., however, only identified a significant age by duration effect of steroids on declarative memory during the first three years of treatment (i.e., the detrimental effects appeared to plateau after the first three years). As none of the Addison's patients in this current study had received treatment with steroids for less than five years, it was not possible to explore

this aspect. Indeed, the significant relationship between duration of treatment and memory performance in the older participants in this current study suggests no plateauing effects at all, at least after the first five years. It is important to note, however, that patients with Addison's disease are treated with replacement levels of steroids and not the same high doses of steroids used to treat other pathologies (e.g., rheumatoid arthritis). Consequently, the effects on memory produced by replacement doses of steroids may be different to those produced by higher doses, at least in the shorter-term.

As described above, both positive and negative relationships were found between age and duration of treatment on memory performance; this suggests inconsistencies in the results produced. More specifically, the significant negative relationship between duration of treatment and total digits forward performance, suggests that long-term treatment with steroids has a beneficial effect on working memory performance (i.e., the longer the treatment the higher the memory score); based on previous research findings this would not have been predicted. In contrast, however, the significant positive relationship between duration of treatment and the total number of detection errors made during the item-recognition task suggests that long-term treatment with steroids can have a detrimental effect on target-detection; based on previous research findings this would have been predicted. In addition, the significant negative relationship between duration of treatment and episodic memory performance using the doors tasks suggests that the longer the treatment the greater the detrimental effects on memory performance, which would also have been predicted.

There is, however, one possible explanation for this discrepancy in results and this relates to previous studies that have investigated the effects of treatment with steroids on patients who have received adrenalectomies. For example, Mitchell & Meaney (1991) found that the administration of steroids given both pre-training and post-training to patients restored the impaired learning behaviour that the adrenalectomy had originally induced. As mentioned previously, as this current study was retrospective in design, information pertaining to the levels of memory performance shown by participants prior to Addison's disease was not available. However, when the participants in this study were asked, generally, how they felt their memory performance had been affected by the diagnosis and treatment for Addison's disease, nearly all of the participants said they felt their memory had got worse.

4.6.2. Effects of other factors on memory performance, irrespective of condition

In addition to the effects of the three different conditions on the different aspects of memory performance, the effects of several other variables, previously shown to affect memory performance, were examined. These included age, IQ levels, levels of caffeine consumed from 24 hours prior to testing, glucose levels and types of food eaten prior to testing. Whilst the effects of each of these variables on performance levels across the three conditions were controlled by using a repeated measures design, some of the effects observed were not expected. The actual results which showed these unexpected effects were those relating to age, IQ, caffeine levels, glucose

levels, food-type, and levels of anxiety and depression. These results are discussed below.

- *Age and memory performance*

The results of this study did not find any significant relationships between age and any of the aspects of memory performance, apart from a significant and positive relationship between age and total scores for the category naming task (rho = 0.695; $p<0.05$). This suggests that an individual's ability to name categories may be enhanced with age and, based on the notion that experience comes with age, these results might have been predicted. In addition to no other significant positive relationships between age and memory, however, there were also no negative relationships. As age-associated deficits in declarative memory have previously only been identified in older-aged adults (e.g., Chiarello & Hoyer, 1988; Light & Singh, 1987) such deficits would not be expected in this younger age group. What wasn't predicted, however, was the lack of any relationship between age and performance on the Spot the Word task. When validating the Spot the Word task, Baddeley et al. (1992) identified a positive correlation between age and performance levels, which the results of this study failed to support (i.e., $r_s = 0.430$; NS).

- *IQ levels (using NART scores) and memory performance*

The IQ levels of participants in this current study were obtained using two different measures. These included the NART and the Spot the Word task. However, whereas previous research identified a positive relationship between the NART and Spot the Word scores (Baddeley et al., 1992), the results of this

study failed to support this (rho = -0.378; NS). There were also no outlying scores to explain this discrepancy. What was also interesting, however, and may be an explanation for this discrepancy in the results was that, whilst Participants 1-4 produced high NART scores, these were not matched by their scores produced by the Spot the Word task (i.e., these scores were much lower in comparison). In contrast, a similar a 'discrepant' relationship was observed for Participants 5-9, who produced lower scores using the NART compared to the higher scores produced using the Spot the Word task. One of the criticisms of the NART is that, 'by requiring participants to read aloud single, unfamiliar words, it is the sort of test which many participants may not have performed since school, almost inevitably results in failure with the most obscure words' and, consequently, can be a source of embarrassment (Baddeley et al., 1992). Indeed, it was this original criticism of the NART which led to the design of the Spot the Word task.

- *Effects of caffeine, glucose and food-type on memory performance*

Although previous research has shown significant effects of each of these three variables on memory performance, no significant effects were found on any of the aspects of memory performance in this study.

- *Effects of depression and anxiety on memory performance*

Levels of depression and anxiety were measured in this study using the BDI and GHQ questionnaires. They were, however, only measured during the first testing session which meant that, as the order of testing conditions was randomised, these measures would have been taken under different conditions

of cortisol. Consequently, as levels of depression and anxiety are sensitive to changes in hormones and cortisol is a steroid hormone. the results produced may have been sensitive to these effects. In addition, during the MRs only condition participants would have been GR deficient. One of the side effects of being GR deficient is feeling generally unwell and lethargic. Consequently, the participants who completed their BDI and GHQ questionnaires during this condition may have been sensitive to these additional effects. In hindsight, therefore, perhaps the BDI and GHQ questionnaires should have either been completed as part of each testing session, or they should have been completed as part of a separate induction session when the participant was taking their normal medication regime. Notwithstanding this, however, the results of this study showed no significant effects of depression levels on memory performance.

The results did, however, find significant relationships between anxiety levels and the scores produced for three of the five working memory tasks (i.e., with total digits backward scores [rho = -0.685; $p < 0.05$]; with total letter naming scores [rho = -0.689; $p < 0.05$]; and with total category naming scores [rho = -0.711; $p < 0.05$]). They also showed significant and negative relationships with two of the four episodic memory tasks (i.e., with total HVLT recall scores [rho = -0.798; $p < 0.05$] and between total scores for the doors task [rho = -0.678; $p<0.05$]), and with one of the two semantic memory tasks (i.e., between total Spot the Word scores [rho = -0.844; $p < 0.01$]). In summary, therefore, the results of this study did show that memory performance (irrespective of condition) can be affected by anxiety levels.

- *BDI scores and GHQ scores*

In contrast to Experiment 2, the results of this study did not find any relationship between depression levels and anxiety levels. The non-significant relationship in this current study, however, was almost significant at r_s = 0.650. This suggests that a significant relationship might have been found if the sample size had been larger, although levels of depression and anxiety in patients with Addison's disease might have been sensitive to the pathology.

- *Effects of being GR deficient.*

As discussed previously, one of the problems during the MRs only condition for patients with Addison's disease is that they would be GR deficient and, consequently, feel less well than normal. Indeed, this may well have been the reason why two of the original eleven participants failed to complete this condition. Nine participants did complete the MRs only condition, however, and although none of these participants reported anything significant at the time, if they had felt 'less well' than normal, it seems reasonable to assume that this may have affected their levels of memory performance. Indeed, although there were no significant differences between perceived levels of stress across the three conditions, the raw data in Figure 25 shows that most of the participants reported higher perceived levels of stress during the MRs only condition, which might have been a reflection of how they were feeling.

4.6.3. Conclusion

Whilst there will always be concerns over using participants from clinical populations, the results of this current study clearly suggest similarities with

those identified by previous research in non-humans. More specifically that individuals generally show better memory performance when both receptors are activated and that the effects produced following activation of the different receptors are related to the stage of memory formation. In conclusion, therefore, the results of this study have extended our understanding of the specific actions of the corticosteroids during memory formation and go some way to support the view that optimal memory function requires balanced activation of the MRs and GRs.

5. Item recognition performance and search strategies

5.1 Abstract

Lupien et al. (1999) interpreted the detrimental effects of acute changes in cortisol levels on item-recognition performance as suggesting that working memory may be more sensitive to the acute effects of cortisol than declarative memory. Two independent studies were carried out to replicate these results. In the first study, the effects of three different acute changes in cortisol levels (i.e., high cortisol vs. control vs. low cortisol) on item-recognition performance were examined in healthy young males at each of two times of day. In the second study, the effects brought about via acute differential activation of the MRs only, GRs only and a combination of MRs/GRs on item-recognition performance were examined in patients with Addison's disease. Whilst the results of both studies failed to show any effects of cortisol on working memory, they did show that participants had responded to the task using the same serial self-terminating cognitive search strategy previously identified by Lupien et al. Taken together, therefore, this suggests that the discrepancy in results were unlikely to have been due to a methodological problem, but due to no significant effects of acute changes in cortisol levels on working memory.

5.2 Introduction

Chapter 1 described how even the slightest differences in experimental factors can make the comparison of results between studies very difficult. For example, the use of different placebos (i.e., glucose vs. saline) and/or having different periods of time between learning and testing (i.e., no delay vs. delay) was put forward to explain the discrepancy in results between the studies carried out by Beckwith et al. (1986) and Kirschbaum et al. (1996). In contrast, Lupien et al. (1999) implicated the use of different encoding instructions (i.e., intentional vs. incidental) to explain the discrepancy in results between their study and the one carried out by Kirschbaum et al. (1996). As described previously, Lupien et al. used intentional encoding instructions and did not find any effects of acute changes in cortisol levels on declarative memory. Kirschbaum et al. however, used incidental encoding (which, as identified by Mandler, 1980 can lead to poorer recall in comparison to intentional encoding) and they did show detrimental effects. As incidental encoding instructions were also used to present the declarative memory tasks in Experiments 2 and 3 (see Chapters 3 and 4) and no detrimental effects were found, this same explanation may explain the discrepancy in results between these results and those identified by Kirschbaum et al.

Another common explanation for discrepancies in results has been the use of different cognitive tasks to measure the same aspects of memory (e.g., De Quervain et al., 2000). Indeed, as described previously, as different memory tasks may 'address' different CNS mechanisms (Lezak, 1983) this makes it difficult for reliable comparisons to be made. Lupien et al. (1999) measured the effects of acute changes in cortisol levels on working memory using an item-recognition task. The same, but

shorter, version of this item-recognition task was also used to measure the effects of acute changes in cortisol on working memory in Experiments 2 and 3.

5.2.1. Sternberg's item-recognition task

The item-recognition task used by Lupien et al. was based upon the work carried out by Sternberg (1966). This has been described in detail elsewhere (e.g., see Lupien et al., 1999). In short, during this task participants are presented with different combinations of between 1 – 4 uppercase letters on a screen (i.e., the target set). After a 750 millisecond delay, participants are then presented with a recognition display (again of 1-4 uppercase letters), which they have to respond to by pressing either the YES or NO buttons. The YES button is pressed when one of the letters included in the recognition display is from the target set; this defines a present-target trial. Alternatively, the NO button is pressed when there are no letters from the target set in the recognition display; this defines an absent-target trial. There is only ever one possible target present in the display on present-target trials.

The task used by Lupien et al. comprised eight different comparison loads (i.e., 2, 3, 4, 6, 8, 9, 12 and 16) made up of 300 trials. The task used in Experiments 2 and 3, however, was a shorter version of this task, comprising five different comparison loads (i.e., 1 vs. 2 vs. 4 vs. 8 vs. 16), made up of 108 trials. As the item-recognition task was only one of a battery of several memory tasks which participants had to complete within a 45 minute period, the reason for reducing the number of comparison load was to reduce the length of the testing session. The time taken to complete the task by Lupien et al. was approximately 30 minutes. In contrast, the time taken to complete the

task in Experiments 2 and 3 was approximately 10 minutes. The dependent variables produced by the item-recognition task included reaction times (in msecs) and the number of detection errors.

As described earlier, Lupien et al. identified significant effects of acute changes in cortisol levels on working memory using this item-recognition task. More specifically, they found that reaction times for the absent-target trials increased significantly at comparison loads of 9, 12 and 16 following the administration of 20 mg hydrocortisone; there were no effects identified following the administration of lower doses of hydrocortisone (i.e., approximately 1.3 mg and 10 mg). In addition, Lupien et al. also interpreted the results of the ANOVA performed on the slopes of the reaction times for the absent-target vs. present-target trials as revealing that the participants had used a serial, self-terminating cognitive search strategy to perform the task.

5.2.2. Serial versus parallel search strategies

Serial memory search is a type of memory search in which information is retrieved one piece after another. Consequently, serial searches are represented by a linear function. That is, when retrieval time is plotted against the number of items to be retrieved the slope of the graph is constant, and is equivalent to the amount of time that it takes to retrieve a single piece of information. In contrast, however, parallel memory search is when a number of pieces of information are retrieved at the same time. Consequently, graphically the slope of the line representing parallel search is zero. That is, as the number of items to be retrieved increases the amount of time that it takes to retrieve these items remains constant. According to Sternberg (1966, 1975)

retrieval from short-term memory relies upon serial type searches, whereas retrieval from long-term memory relies upon parallel type searches.

To identify whether the discrepancy in results found by Experiments 2 and 3 compared to those found by Lupien et al. might be explained by a difference in the way participants had responded to the task, these data were explored in more detail. If the analysis showed that participants had responded using a different search strategy to that identified by Lupien et al., then this suggests that the discrepancy in results points to a methodological problem. However, if the analysis showed that participants had responded using the same search strategy, then this suggests more strongly that the data had, indeed, shown no effects of acute changes in cortisol levels on working memory. The results produced for Experiment 2, which was carried out on healthy young adults and a similar type of target population used by Lupien et al. (i.e., students) are presented first. The results for Experiment 3, which was carried out using Addison's patients, follow.

5.3 Results

5.3.1. Results for Experiment 2.

As described in Chapter 3, the purpose of this study was to investigate the effects of acute changes in three conditions of cortisol levels (i.e., high cortisol vs. control vs. low cortisol) and time of day (i.e., morning vs. afternoon) on memory performance. The mean reaction times for each condition and at both times of day are shown in Figure 26. These show a lack of any significant time of day effects. In addition, the mean reaction times for each type of task (i.e., present-target vs. present-absent) and for each comparison load (i.e., 1 vs. 2 vs. 4 vs. 8 vs. 16) at both times of day (i.e., am vs. pm) are shown in Figures 27 - 30.

Figure 26 : Mean reaction times produced for each condition at both times of day.

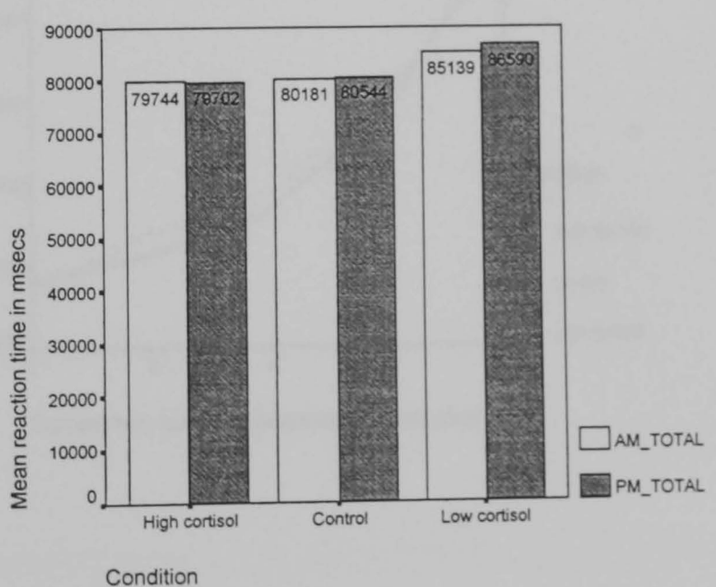


Figure 27 : The effects of condition on group mean reaction times for present-target trials in the morning.

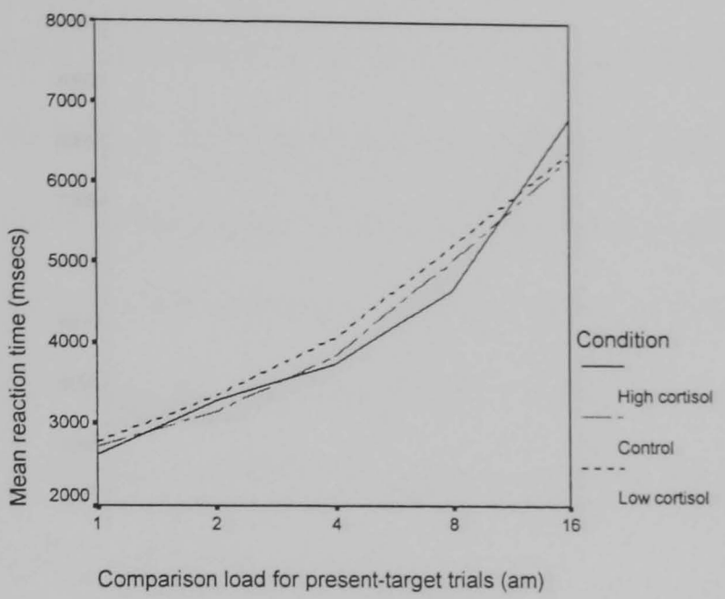


Figure 28 : The effects of condition on group mean reaction times for present-target trials in the afternoon

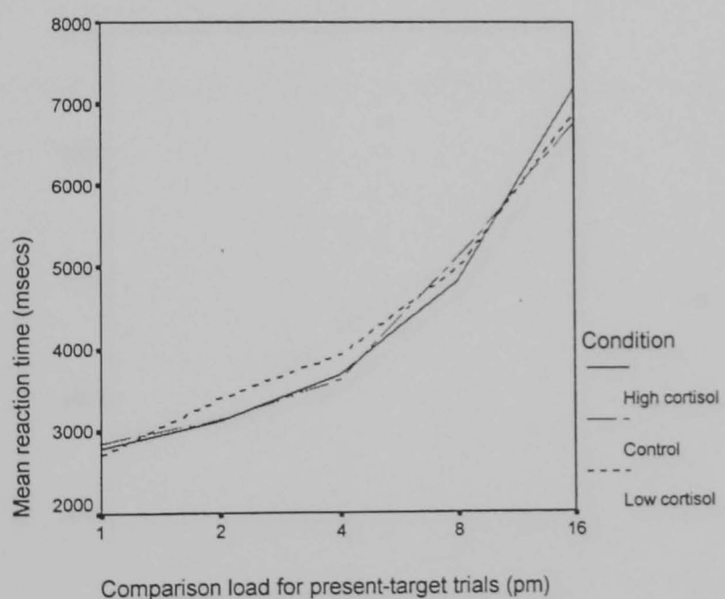


Figure 29 : The effects of condition on group mean reaction times for absent-target trials in the morning

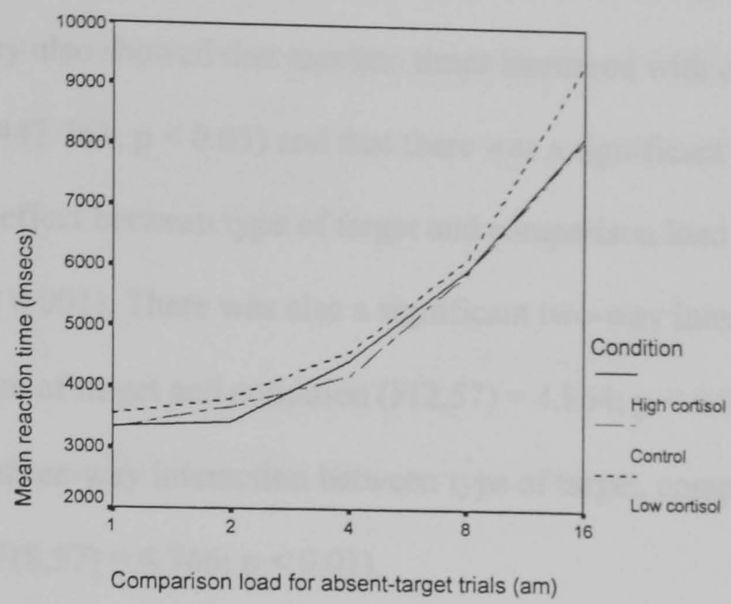
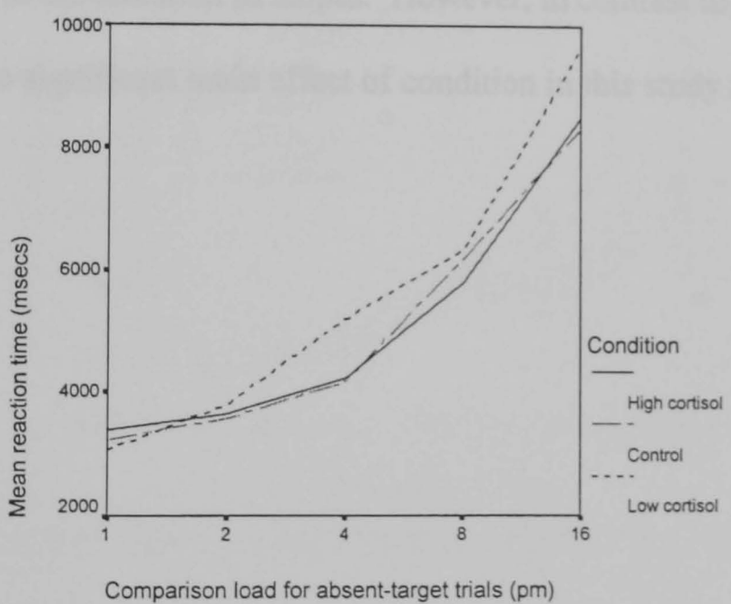


Figure 30 : The effects of condition on group mean reaction times for absent-target trials in the afternoon



A four-factor (2 x 5 x 2 x 3) mixed ANOVA, with type of target (i.e., present-target vs. absent-target), comparison load (1 vs. 2 vs. 4 vs. 8 vs. 16), and time of day as the three within-group factors, and condition as the between-groups factor was carried out on the mean reaction times. Whilst these results showed no significant difference in reaction times as a function of cortisol levels ($F(2,57) = 1.418$; NS) or time of day ($F(1,57) = 0.322$; NS),

they did show that participants took longer to complete the trials when a target was absent as opposed to when one was present ($F(1,57) = 245.481$; $p < 0.001$) They also showed that reaction times increased with comparison load ($F(4,57) = 447.763$; $p < 0.05$) and that there was a significant two-way interaction effect between type of target and comparison load ($F(4.57)= 36.710$; $p < 0.001$). There was also a significant two-way interaction effect between type of target and condition ($F(2,57) = 4.864$; $p < 0.05$) and a significant three-way interaction between type of target, comparison load and condition ($F(8,57) = 4.766$; $p < 0.01$).

A comparison of the reaction-time graphs with those produced by Lupien et al. for one time of day only (see Figures 31 and 32) also show similarities in the direction of slopes. However, in contrast to Lupien et al., there was no significant main effect of condition in this study at either time of day.

Figure 31 : Showing the effects of condition on group mean reaction times for present-target trials identified by Lupien et al. (1999)

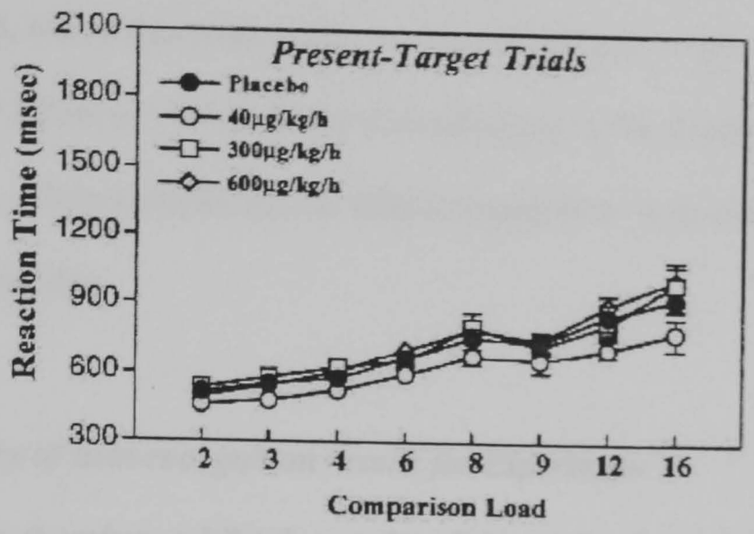
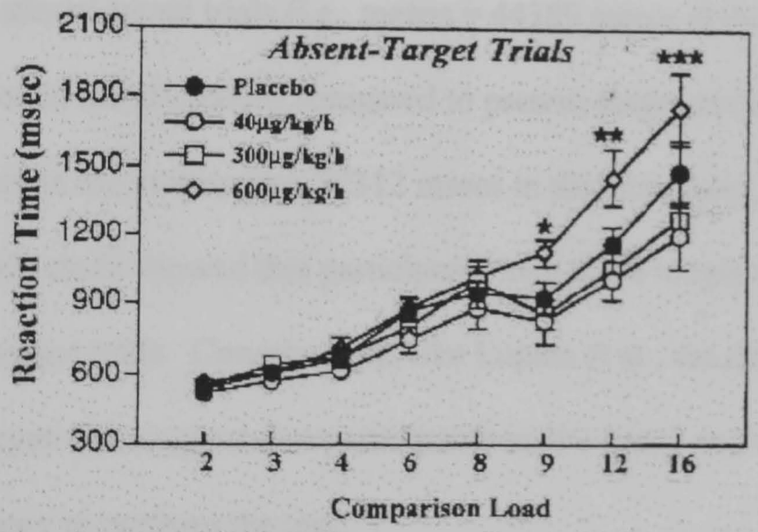


Figure 32 : Showing the effects of condition on group mean reaction times for absent-target trials identified by Lupien et al. (1999)



A post hoc analysis, using Tukeys, was carried out on the main effect of comparison load. This showed significant differences between each of the comparison loads with each other (i.e., $p < 0.001$) and at both times of day (i.e., $p < 0.001$). Like Lupien et al., a post-hoc analysis was also carried out breaking the three-way interaction (i.e., condition by comparison load by time of day) down by target type. This revealed a significant condition by comparison load interaction for the absent-target trials only ($F(8,228) = 2.353$; $p < 0.05$), but not for present-target trials ($F(8,228) = 1.431$; NS). A further

post-hoc analysis carried out to compare the time of day effects on the significant condition by comparison load interaction for absent-target trials showed that, whilst this relationship was still significant in the afternoon ($F(8,228) = 2.236$; $p < 0.05$), it was not significant in the morning ($F(8,228) = 1.221$; NS). More specifically, the effects appeared to have been influenced by the time of day.

- *Summary of item-recognition results for Experiment 2*

In summary, therefore, whilst the results of this study showed no significant main effects of acute changes in cortisol levels on item-recognition performance, they did show that participants took significantly longer to respond to absent-target trials (i.e., means = 44389 msecs in the morning vs. 44966 msecs in the afternoon) compared to present-target trials (i.e., means = 37299 msecs in the morning vs. 37312 msecs in the afternoon). Indeed, the results of this study showed that participants took 20 % longer to respond to the absent-target trials. Consequently, like Lupien et al., the data for reaction times by target-type suggest that participants used a serial, self-terminating search strategy to perform the task.

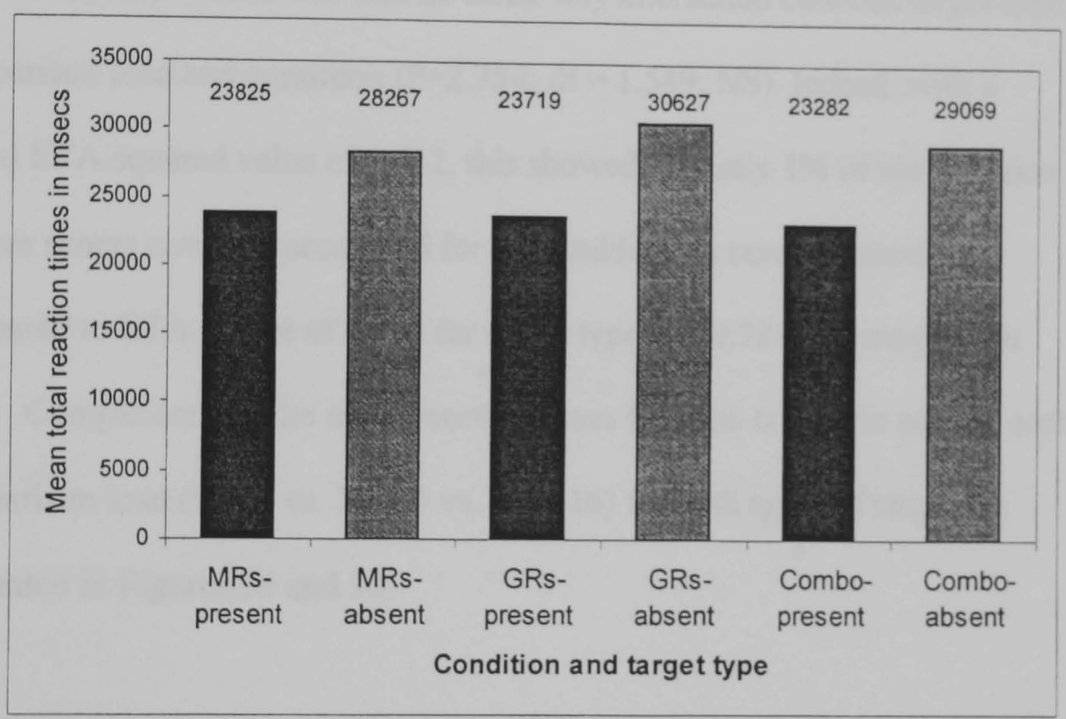
5.3.2. *Results for Experiment 3*

As described in Chapter 4, the purpose this study was to investigate the effects of different acute changes in cortisol on memory performance following activation of the different corticosteroid receptors using steroids.

- *Effects of condition on mean reaction times*

The mean reaction times for item-recognition performance for each type of task (i.e., present-target vs. absent-target) and for each condition (i.e., MRs only vs. GRs only vs. GRs/MRs) are shown in Figure 33. This also shows the reaction times produced during the present-target trials compared to the absent-target trials.

Figure 33 : Mean reaction times for each condition and for each type of trial



As for Experiment 2, the results of this study showed that participants took longer to complete the trials when a target was absent than when a target was present. The scores for total reaction times were not normally distributed. However, as the assumptions for homogeneity of variance and sphericity were met a repeated measures three-factor ANOVA, with target-type (i.e., present-target vs. absent-target), comparison load (i.e., 1 vs. 2 vs. 4 vs. 8 vs. 16), and condition as the three factors was carried out on the data. Whilst the results

showed no main effect depending on which type of corticosteroid was activated ($F(2,12) = 0.075$; NS), they did show that participants took significantly longer to complete the trials when a target was absent as opposed to when one was present ($F(1,6) = 15.150$; $p<0.01$). They also showed that reaction times increased with comparison load ($F=33.726$; $df = 1.225$; $p < 0.001$). There were, however, no significant two-way interactions between: target-type and comparison load ($F=4.168$; $df = 1.340$; NS); target-type and condition ($F(2,12) = 0.185$; NS) or comparison load and condition ($F=0.783$; $df = 3.148$; NS). There was also no three-way interaction between target-type, comparison load and condition ($F=2.754$; $df = 1.549$; NS). Indeed, with a partial ETA squared value of 0.012, this showed that only 1% of the variation in error scores could be accounted for by condition on reaction times, compared to ETA values of 0.884 for target type and 0.721 for comparison load. Comparisons of the mean reaction times for each condition and for each comparison load (i.e., 1 vs. 2 vs. 4 vs. 8 vs. 16) for both types of target are illustrated in Figures 34 and 35.

Figure 34 : Showing the effects of condition on mean reaction times for target-present trials at each comparison load

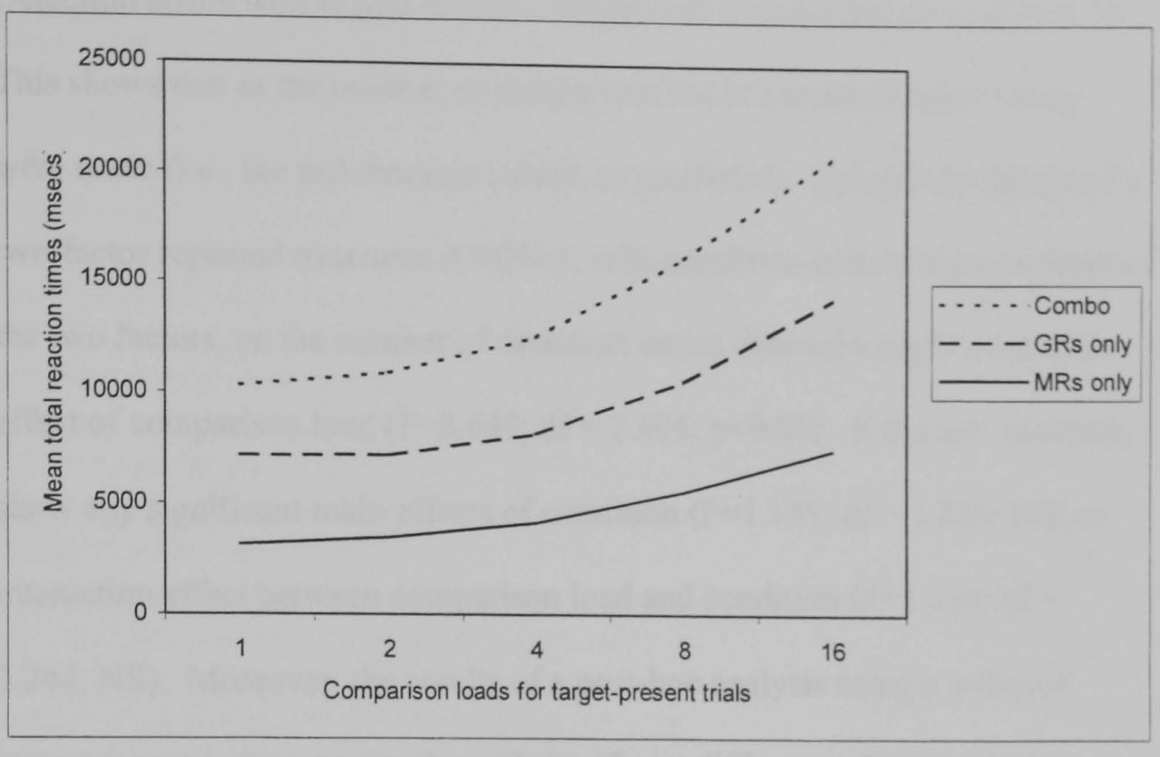
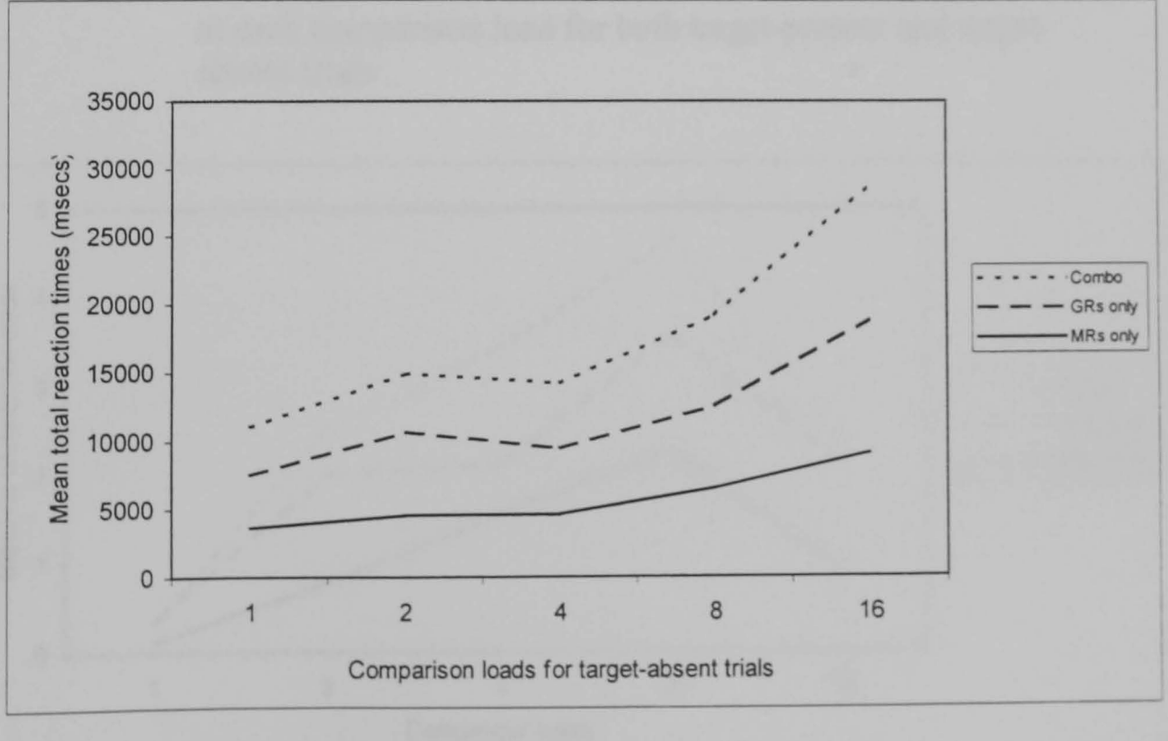


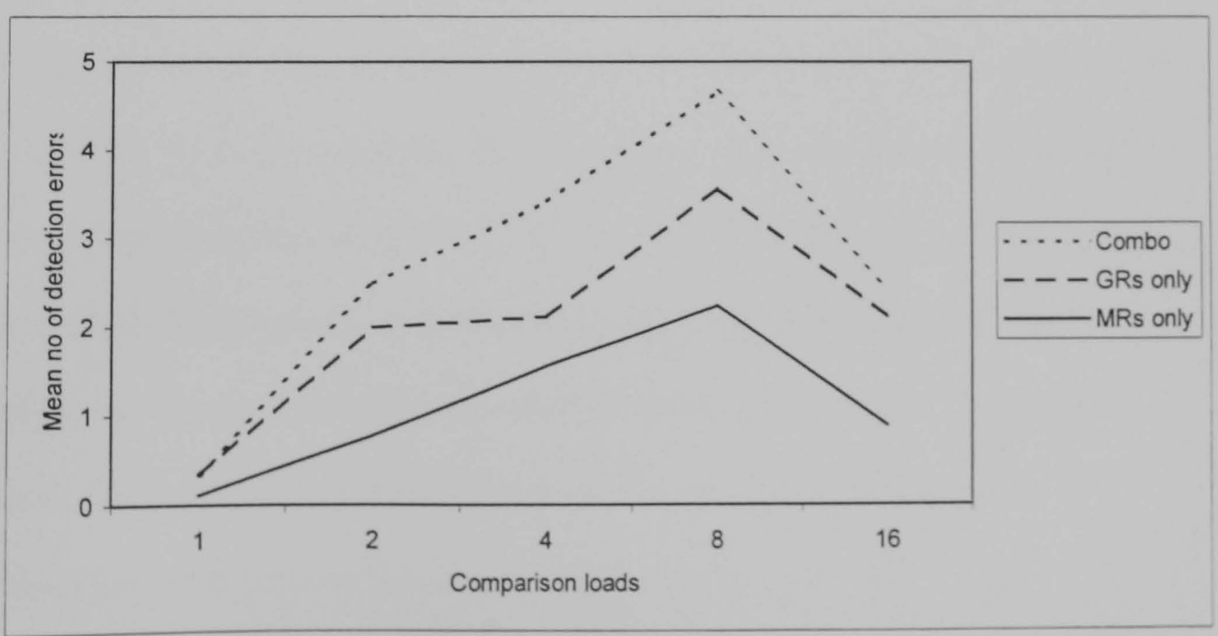
Figure 35 : Showing the effects of condition on mean reaction times for target-absent trials at each comparison load



- *Effects of condition on number of detection errors*

Detection errors with regard to each comparison load are shown in Figure 36. This shows that as the number of comparison loads increased, more errors were made (i.e., the task became harder, as predicted). Indeed, the results of a two-factor repeated measures ANOVA, with condition and comparison load as the two factors, on the number of detection errors showed a significant main effect of comparison load ($F=3.640$; $df = 2.494$; $p<0.05$). It did not, however, show any significant main effects of condition ($F=1.139$; $df = 1.224$; NS) or interaction effect between comparison load and condition ($F=1.234$; $df = 3.244$; NS). Moreover, the results of a post-hoc analysis using a series of pairwise comparison t-tests showed significant differences between comparison load 1 with those produced by comparison loads: 4 ($p<0.05$); 8 ($p<0.01$) and 16 ($p=0.001$) only.

Figure 36 : Showing the effects of condition on mean no of detection errors at each comparison load for both target-present and target-absent trials



This shows that, in all three conditions, the number of detection errors dropped after comparison load 8.

- *Summary of item-recognition results for Experiment 3*

In summary, therefore, whilst the results of this study showed no significant main effects of acute levels of cortisol on item-recognition performance as a result of different receptor activation, they did show that participants took significantly longer to respond to absent-target trials (i.e., mean = 86950 msecs) compared to present-target trials (i.e., mean = 70757 msecs). Indeed, as for Experiment 2, they showed that participants took 20 % longer to respond to the absent-target trials. Consequently, like Lupien et al., the data for reaction times by target-type suggest that participants used a serial, self-terminating search strategy to perform the task.

5.4 Discussion

As described earlier, the primary aim of this chapter was to present the results of Experiments 2 and 3 that were produced using the same item-recognition task as that used by Lupien et al. (1999). More specifically, the rationale for doing this was to identify whether the discrepancy in results concerning the lack of effects of acute changes in cortisol identified by Experiments 2 and 3 compared to the detrimental effects identified by Lupien et al. points to a methodological problem. However, the results suggest that, as for Lupien et al., the participants had responded to the task using a serial self-terminating cognitive search strategy. Consequently, this shows that the discrepancy in results could not be explained by a difference in the way participants had responded to the task and more strongly suggests that the results showed that no effects of cortisol on working memory were found. Moreover, the results of these two studies support Sternberg's claim that retrieval from short-term memory relies upon serial type searches (e.g., 1966, 1975).

The participants in Experiments 2 and 3 were from different target populations (i.e., healthy adults versus a clinical population). However, the results produced by both studies showed that participants took 20 % longer to complete target-absent trials compared to the target-present trials. Taken together, therefore, these results suggest that chronic treatment with steroids (i.e., for the Addison's patients) does not affect how individual's search for items in short-term memory. The comparison of results produced at two times of day also suggests that, whilst the type of search strategy used may not be affected by the time of day per se., the effects of condition by comparison load identified for the absent-target trials may have been. More specifically, the results of Experiment 2 showed that whilst there was still a

significant condition by comparison load interaction in the afternoon, it was not significant in the morning.

One important question raised by these results, therefore, is why did participants take longer to respond to the absent-target trials compared to the present-target trials? Very simply, this can be explained by the fact that individuals had used a self-terminating search strategy as opposed to an exhaustive search strategy. According to Sternberg (1975), during an exhaustive search the target item is compared to each member of the recognition set before a decision is made on whether a match has been found, even if a match actually occurs early in the comparison process. In contrast, a self-terminating search stops whenever a match occurs. During an absent-target trial, the individual has no choice but to search exhaustively (i.e., there is no match to be made). However, during a present-target trial, the individual is given a cut off point (i.e., the match) at which to stop searching. As the results of Experiments 2 and 3 both showed that participants took 20 % significantly longer to complete the absent-target trials compared to the present-target trials, this suggests that participants in both studies were using a serial, self-terminating search.

In conclusion, therefore, whilst the use of different cognitive measures, testing procedures and/or target populations may have been used by previous researchers to explain discrepancies in results, this is not an appropriate explanation here. Indeed, the results of Experiments 2 and 3 suggest that the discrepancy in results concerning the effects of acute changes in cortisol levels on working memory were unlikely to have been due to a methodological problem.

6. Conclusions

6.1. Introduction

The overall aim of this thesis was to explore the effects of acute changes in cortisol levels on working memory and the episodic and semantic components of declarative memory in humans, and to summarise and interpret the data obtained following previous investigations, and then identify and explore some of the factors which, at that point, remained unclear. These included the immediate effects of acute changes in cortisol levels (both increased and decreased) on working memory and declarative memory; the additional effects of time of day; and the effects on memory brought about following different activation of the MR and GR corticosteroid receptors. In addition to furthering our current knowledge concerning the effects of cortisol on memory, one of the main reasons for exploring these factors was to further investigate the inverted U-shaped relationship between corticosteroids and memory which has been shown to modify the magnitude and direction of effects produced.

Detailed discussions of the data produced by each study have been presented. However, the purpose of this final chapter is to summarise and evaluate these data from a more global perspective and see whether the literature reviewed in Chapter 1 may now be interpreted in a different way. This chapter will also introduce the findings of more recent research, published since Experiments 2 and 3 were carried out, which presents a fundamental shift in thinking about the effects of corticosteroids on memory. The results of both experiments will also be evaluated in the light of this.

As Experiments 2 and 3 were carried out using different populations (i.e., non-clinical versus clinical), to measure the effects of acute changes in cortisol levels produced by different methods of manipulation (i.e., different levels of cortisol versus

those produced by activation of the different corticosteroid receptors), a summary of the results of each study will first be presented separately. A summary and interpretation of the results from a more global perspective will follow, including some suggestions for the directions of future research.

6.2. Experiment 2

6.2.1. Summary of the aims and findings

As described in Chapter 1, whilst a review of the previous research clearly identified several factors which have been shown to modify the effects of cortisol on memory, the effects of several other potential factors remained unclear. The aim of Experiment 2 was to address some of these factors. These included the effects of: acute changes in cortisol levels on working memory and declarative memory; time of day; significantly reduced levels of cortisol; and acute changes in cortisol levels on the episodic and semantic components of declarative memory. The relationship between perceived levels of stress and cortisol levels was also explored. A summary of the results obtained follow.

- *The effects of acute changes in cortisol levels on working memory and declarative memory*

Chapter 1 describes how, compared to the effects of chronic changes in cortisol levels, the effects of acute changes in cortisol levels on memory remain less clear. The common finding is that chronic elevations impair declarative memory (Lupien et al., 1997, 1994; Newcomer et al., 1994; Seeman et al., 1997), whereas the effects produced following acute elevations

have been mixed. For example, Kirschbaum et al. (1996) found that declarative memory was impaired one hour following the acute administration of 10 mg hydrocortisone, however Lupien et al. (1999) found no effects at all.

As a result of initial observations of the effects of cortisol on rodent brains, the majority of previous research has also focused primarily on the 'stress-hippocampus link' (Lupien & Lepage, 2001). Consequently, in comparison to the effects on the hippocampal forms of memory (i.e., declarative/explicit memory) the effects of acute and chronic changes in cortisol levels on working memory are also less clear. Indeed, at the time of writing this thesis, Lupien et al. (1999) were the only researchers to suggest that working memory may be more sensitive to acute changes in cortisol levels; they found no effects on declarative memory at all.

One of the aims of Experiment 2 was to address this disparity. The effects of acute changes in cortisol levels (both high and low) were investigated on working memory and the episodic and semantic components of declarative memory. However, whilst the results (like Lupien et al.) showed no effects of acute changes in cortisol levels on declarative memory, in contrast to Lupien et al. they also showed no effects of acute changes in cortisol levels on working memory.

As mentioned previously, at the time of writing this thesis the effects on working memory identified by Lupien et al. had not been replicated. Consequently, the results of Experiment 2 suggest that the effects of acute changes in cortisol levels on working memory remain unclear. However, the results of a more recent study by Wolf, Convit, McHugh et al. (2001) have since shown that acute changes in cortisol levels, produced following the

intravenous administration of 0.5 mg/kg cortisol, impaired working memory performance (using a digit span task) in young males (mean age = 24 ± 1.2 years) thirty minutes later; no such effects were identified in the elderly males (mean age = 69 ± 1.8 years). According to Wolf et al., the lack of cortisol 'responsivity' identified in the elderly males may be explained by the 'age-related alterations of the frontal cortex'; this suggests that the effects of acute changes in cortisol on working memory may be dependent on age. The participants in the study by Lupien et al. were also young and this suggests that, in comparison to older adults, the young are more sensitive to the effects of acute changes in cortisol levels on working memory. The detrimental effects on working memory identified by Wolf et al. were also more pronounced during a second testing phase given almost three hours after cortisol administration. This suggests that acute changes in cortisol levels might have suppressed the practice effect.

Whilst the results of the study by Wolf et al. appear to lend support to those identified by Lupien et al., the differences in measures used to identify the effects requires consideration. The detrimental effects on working memory identified by Wolf et al. were measured in digit performance. In contrast, however, both Lupien et al. and Experiment 2 used the same item-recognition task. As described in Chapter 1, the use of different cognitive measures to assess the same aspects of memory makes it difficult for reliable comparisons across studies to be made (De Quervain et al., 2000). Moreover, as digit span performance is also regarded as a measure of attention (see Lezak, 1995), there is still some dispute as to whether this can be regarded as a pure measure of working memory. The effects of acute changes in cortisol

levels on digit span performance were also measured in Experiment 2. However, in contrast to Wolf et al., no effects of acute changes in cortisol levels were found.

The results of the study by Wolf et al., together with those identified by Experiment 2, do, however, suggest that the magnitude and/or direction of the effects of corticosteroids on human memory can depend on the time of testing relative to learning (e.g., De Quervain et al., 2000). Wolf et al. found that acute increases in cortisol levels given one hour pre-learning had no effect on the learning or recall of declarative information. The administration of hydrocortisone to increase cortisol levels in Experiment 2 was given 2-3 hours before learning and, as a similar lack of effects on declarative memory was found, these results further suggest that acute changes in cortisol levels may specifically impair retrieval (De Quervain et al., 2000). They also suggest that the administration of hydrocortisone given immediately after presentation of a word list affects immediate recall, but not delayed recall. In conclusion, therefore, as identified by Wolf et al., this suggests that the 'recall of material learned under normal cortisol levels is impaired by high cortisol levels, but the recall of material learned while cortisol levels are high may not be influenced by high cortisol levels' (p.1007). Specifically, that an effect of corticosteroids on memory may only occur if the levels of cortisol at learning and testing are different. Unfortunately, although previously identified in rats (De Quervain et al., 1998), this information was not available for consideration when Experiment 2 was carried out. Consequently, as the design of Experiment 2 meant that the levels of cortisol in the high cortisol condition were high during both learning and testing, according to this interpretation, no effects would be

expected. As suggested by Wolf et al. (2001), future studies comparing the effects of cortisol levels altered before learning and before recall testing with those produced when cortisol levels are altered before recall testing only, need to be carried out to test this hypothesis.

Taken together, therefore, whilst the results of the study by Wolf et al. go some way to suggest that working memory, at least in younger adults, may be more sensitive to acute changes in cortisol levels than declarative memory, further studies still need to be carried out to support this. Further studies also need to be carried out looking at the effects of corticosteroids on the memory functions associated with other regions of the brain (e.g., the frontal and pre-frontal cortices). This is based on new findings which suggest that the idea of learning and memory as comprising a single entity (a concept used by the majority of studies) may actually be 'a composite of various cognitive processing components that are also distributed in different regions of the brain' (Lupien & Lepage, 2001, p. 51). More recent data also suggests that, whilst there are similarities between rodent and human brains, there are distinct phylogenetic differences in their development which suggest that memory does not equal hippocampus in humans (Lupien & Lepage, 2001). For example, whilst the subcortical structures play a more important role in cognitive function in rodents, the development of these cortical areas in humans leads to stronger involvement. The same authors also suggest that the differences in effects of corticosteroids on memory in rodents compared to humans might be explained by the 'preferential distribution and affinity' of the different corticosteroid receptors. Consequently, this suggests that future research should place greater emphasis on the effects on memory produced

following this differential activation of these receptors when studying the effects of stress hormones on the brain, which is something Experiment 3 was designed to address.

Evidence using immunohistochemistry report also suggests that the primate's brain is not the major site for the expression of corticosteroids (Leverenz, Wilkinson, Raskind & Peskind, 1999; Ongur & Price, 1997). Taken together, therefore, this more recent shift in thinking suggests that previous studies which have focused on the effects of corticosteroids on the hippocampus only to the detriment of other brain regions, might have 'missed the opportunity to identify the real actions of stress hormones on the brain' (Lupien & Lepage, 2001). There also might be potential flaws to the conclusions that have, thus far, been reached.

- *Effects of time of day*

A second factor made apparent by a review of the literature was how little is known about the additional effects of time of day on the relationship between cortisol and memory performance. Whilst this had been investigated by Fehm-Wolfsdorf et al. (1993), the majority of previous researchers have either not reported or controlled for the potential effects of time of day. For example, by testing participants at the same time of day (e.g., Kirchbaum et al., 1996; Lupien et al., 1999; Newcomer et al., 1994). As described in Chapter 1, Fehm-Wolfsdorf et al. tested the effects of acute elevations in cortisol levels at each of two times of day (i.e., at 09.00 hrs and at 18.00 hrs). They found no significant differences in declarative memory performance as a function of cortisol levels, but memory performance of participants in the

placebo group was enhanced in the morning. The administration of hydrocortisone to increase cortisol levels appeared to suppress this peak in performance in the morning. In an attempt to gain some further insight into the inverted U-shaped relationship between cortisol and memory performance and the effects of time of day, the design of Experiment 2 was very similar to that used by Fehm-Wolfsdorf et al. The effect of acute changes in cortisol levels (both high and low) on working memory and declarative memory were tested at two times of day (i.e., at 09.00 or 10.00 hrs, and at 17.00 hrs). Moreover, like Fehm-Wolfsdorf et al., no differences in declarative memory, or working memory, were identified as a function of cortisol levels. However, in contrast to Fehm-Wolfsdorf et al., Experiment 2 also found no differences in either aspect of memory performance as a function of time of day. Rather, the mean levels of memory performance for each of the three groups and at both times of day were all very similar. Consequently, this suggests that the inverted U-shaped relationship between cortisol levels and memory performance may be different following acute changes in cortisol levels compared to that produced following chronic changes.

The results of a more recent study by Lupien, Wilkinson, Briere et al. (2002), however, suggest that the inverted U-shaped relationship between acute changes in cortisol levels and memory performance are similar to those produced following chronic changes. Lupien et al. investigated the effects of a bolus injection of 35 mg hydrocortisone on recognition performance in young, healthy males when administered in the morning (during the 'circadian peak') compared to the afternoon (during the 'circadian trough'). On both occasions memory performance was tested five minutes following the administration of

hydrcortisone. When administered in the morning, the acute increase in cortisol levels had a negative effect on word-stem recognition performance. However, when administered in the afternoon, recognition performance was enhanced.

The effects of acute increases in cortisol levels on recognition performance during the circadian trough were also investigated in Experiment 2 but, in contrast to Lupien et al., no effects on recognition performance were found. However, whereas Lupien et al. reported a positive effect on cognitive performance in the afternoon, they did not find a difference in recognition performance levels per se. Rather, in the afternoon participants in the hydrocortisone group responded significantly faster for correct trials when compared to the placebo group. Reaction time for recognition performance was not measured in Experiment 2; indeed, participants were told explicitly at the start of the Names and the Doors recognition tasks that response times were not being measured. Consequently, this difference in cognitive measures makes it difficult to compare results.

- *Effects of significantly reduced levels of cortisol*

A review of the literature also showed that, whilst previous researchers have investigated the effects of increased levels of cortisol on memory, the effects of significantly reduced levels of cortisol were unclear. Accordingly, as previous research suggests that acute periods of controllable stress can be beneficial because of the effect on arousal levels (e.g. Epel et al., 1998), the effects of significantly reduced levels of cortisol on memory performance were investigated in Experiment 2. The levels of endogenous cortisol for

participants in the low cortisol group were reduced by the administration of metyrapone, a cortisol inhibitor which blocks the synthesis of cortisol. However, even though previous research suggests that a certain degree of GR activation appears to be a pre-requisite for the long-term storage of information (De Quervain et al., 1998) and the inverted U-shaped relationship between cortisol and memory performance suggests that memory performance may be impaired by levels of cortisol which are too low (Lupien & McEwen, 1997), no differences in any aspect of memory performance as a function of significantly reduced cortisol levels were found. This suggests that, at least in the short-term, memory performance is not affected when cortisol levels are significantly reduced. Alternatively, in the same way as for acute increases in cortisol, the lack of any difference between reduced levels of cortisol during learning and testing may explain this lack of effect. Future studies, therefore, need to compare the effects of levels of cortisol which are reduced before learning and before recall testing, with those produced when cortisol levels are reduced before recall testing only.

Since Experiment 2 was carried out, however, Lupien et al. (2002) have also looked at the effects of significantly reduced levels of cortisol on declarative memory performance and, like Experiment 2, administered metyrapone orally to decrease cortisol levels. In contrast to Experiment 2, Lupien et al. found that treatment with metyrapone significantly impaired free recall performance. However, in contrast to Experiment 2, which only looked at the effects of metyrapone on immediate free recall performance, Lupien et al. also investigated the effects on delayed recall (i.e., the effects were measured 20 minutes later). Furthermore, whilst Lupien et al. did identify

detrimental effects of metyrapone treatment on delayed recall performance. they did not identify any effects of metyrapone on the first three learning trials (i.e., immediate recall performance). Consequently, the results found by Lupien et al. suggest a similar lack of effects of metyrapone on immediate free recall performance as those identified by Experiment 2.

- *Effects of acute changes in cortisol levels on the episodic and semantic components of declarative memory*

The primary reason for exploring the effects of acute changes in cortisol levels on the episodic and semantic components of declarative memory was to identify whether the two components of declarative memory are unitary (e.g., Cohen et al., 1997), or partly dissociated (e.g., Vargha-Khadem et al., 1997). Moreover, whether both components of declarative memory are affected to a similar degree by changes in cortisol levels and if so, whether this suggests that both episodic and semantic memory are similarly dependent on the integrity of the hippocampus. The results of Experiment 2, however, failed to find any difference in either episodic or semantic memory performance as a function of cortisol levels or time of day. As previous research suggests that declarative memory is impaired by chronic changes in cortisol levels, a study looking at the effects of chronic changes on the episodic and semantic components of declarative memory separately might produce different results. In the meantime, however, it still remains unclear whether both components of declarative memory are affected to a similar degree by acute, or chronic, changes in cortisol levels.

- *Perceived levels of stress and cortisol levels*

As described in Chapter 1, the results of previous studies that have looked at the relationship between perceived levels of stress and cortisol levels have been mixed. For example, whereas Lupien et al. (1998) identified a significant and positive relationship between perceived levels of stress and cortisol, De Quervain et al. (2000) did not. Indeed, Vedhara et al. (2000) found that those students who reported the highest perceived levels of stress showed lower levels of cortisol. In contrast to Vedhara et al., the results of Experiment 2 showed a significant and positive relationship between cortisol levels and perceived levels of stress in the afternoon, as well as a non-significant but high ($r = 0.6$) correlation in the morning. Experiment 2, however, did not find a significant relationship between perceived levels of stress and memory performance at either time of day. Moreover, as discussed in Chapter 3, whether it is the levels of cortisol per se, which determine how stressed an individual perceives themselves to be or vice versa, or whether it is an interaction between the two and/or other factors, remains unclear.

- *Anxiety levels and cortisol-response*

Perhaps one of the most interesting observations made during Experiment 2 was the significant differences in cortisol-response produced, both as a function of condition, as well as between high- and low cortisol responders within each condition. Moreover, these significant differences in cortisol-response occurred irrespective of whether the acute changes in cortisol levels were manipulated using medication (i.e., in the high and low cortisol groups), or as a result of time of day (i.e., in the control group).

As described in Chapter 1, Brown et al. (1996) suggested that individual differences in cortisol-response may be positively related to anxiety levels. Indeed, although the levels of anxiety were only obtained during the induction phase of Experiment 2 (and not on each testing day when cortisol levels had been manipulated), the results showed a significant and positive relationship between anxiety levels and cortisol-response. Consequently, these results go some way to support the interpretation made by Brown et al. A post-hoc analysis comparing memory performance between high- and low-responders within each condition also showed no effects on either aspect of memory performance as a function of acute changes in cortisol levels or time of day. This further suggests that there were no effects of acute changes of cortisol on memory performance.

6.2.2. *Summary of the findings*

In summary, therefore, like several other previous researchers the results of Experiment 2 have shown that declarative memory is not affected by acute changes in cortisol levels. Thus, this suggests that the effects on declarative memory produced following chronic changes in cortisol levels may be very different to those produced following acute changes. It also suggests that any differences in the effects on the episodic and semantic components of declarative memory may only be identifiable following chronic changes in cortisol levels. In line with a more recent interpretation of the research (i.e., that reported by Lupien & Lepage, 2001) it also suggests that the effects of acute changes in cortisol on memory does not necessarily equal hippocampus in humans. In contrast to Lupien et al. (1999) and, more recently, Wolf et al.

(2001) however, the results of Experiment 2 also do not show that working memory is more sensitive to acute changes in cortisol levels than declarative memory. Moreover, as this lack of effects on working memory was identified using the same cognitive measure as Lupien et al., the discrepancy does not point to methodological differences and further suggests that a lack of acute changes in cortisol levels on working memory were found. As previous research has shown that acute changes of very high levels of cortisol impair declarative memory performance (Newcomer et al., 1999), this suggests that it may "take several days of stresses like major surgery or severe psychological trauma in order for cortisol to produce memory impairment" (p.352).

Perhaps one of the most significant contributions made by Experiment 2 is that it has reinforced the importance of considering differences in methodology when comparing results between studies. As described in Chapter 1, the effects of cortisol on memory performance appear highly sensitive to even the most subtle differences in methodology. This, together with the more recent findings by De Quervain et al. and Wolf et al. suggesting that the effects of acute changes in cortisol on memory depend on when these changes occur in relation to learning and testing, is something future research now needs to address. Indeed, Wolf, et al. (2001) have already suggested that "stress exposure between learning and the recall phase in contrast to stress exposure before learning could have led to different results" (p.717; Lupien et al., 1997; Wolf et al., 1999).

A second significant contribution relates to how little effect, even large significant differences in cortisol levels (i.e., between the three conditions, as well as between high- and low responders within each condition) had on

memory performance. Moreover, the results of Experiment 2 suggest that, whereas changes in cortisol levels may be regarded as an objective measure of stress (Kirschbaum, Prussner et al., 1995), they cannot be considered an objective index of memory performance. As previously identified by Kirschbaum et al. (1992), individual interpretation appears to play a much greater part in the effects of cortisol on memory than simply a change in cortisol levels per se. Indeed, as research looking at the relationship between perceived levels of stress and job satisfaction has shown, contrary to what theory predicts, those individuals reporting the highest levels of stress do not always report the lowest levels of job satisfaction (e.g., Cox, 1993). The individual's appraisal of the effects of stress, along with other factors such as personality and locus of control, appear to determine the effects produced.

6.3. Evaluation of Experiment 3

6.3.1. Summary of the aims and findings

The primary aim of Experiment 3 was to investigate the claim that the selective effects of corticosteroids on memory performance may be attributed to the differential activation of the MRs and GRs. As previous research exploring this theory has only been carried out in non-primates, the purpose of Experiment 3 was to see if activation of the MRs and GRs (using different types of steroids) affect different aspects of memory processing in humans (e.g., Oitzl & De Kloet, 1992). The purpose was also to see whether balanced activation of both the MRs and GRs is, indeed, necessary for optimal memory performance (De Kloet et al., 1999).

The sample of participants used in the study were patients with Addison's disease. These patients are treated with replacement levels of cortisol throughout life. Consequently, Experiment 3 also provided an opportunity to examine the effects on memory produced following chronic treatment with steroids and to examine whether the effects produced are modified by treatment duration. In addition to increasing our understanding of the effects of corticosteroids on memory, the results produced may also have implications for the effects of steroid-therapy on memory.

A summary of the results showed that, although not consistent across all memory tasks, participants showed poorer working memory performance when the GRs only were activated. In contrast, they showed poorer episodic memory performance when the MRs only were activated. Moreover, during both tasks, participants produced the highest scores when both receptors were activated, which suggests that balanced activation of MRs and GRs is necessary for optimal memory function. These results extend and support those found previously in rats and chickens, using receptor agonists and antagonists (e.g., Oitzl & De Kloet, 1992; Sandi & Rose, 1994).

According to the more recent interpretation of results reported by Lupien & Lepage (2001), the preferential distribution and affinity of MRs and GRs throughout the brain may explain the selective effects on memory produced. The results of Experiment 3 go some way to support this. For example, these results showed that working memory is more sensitive to the detrimental effects of high levels of GRs and/or no MRs (i.e., participants showed poorer working memory performance when the GRs only were activated). Whilst the frontal cortex contains both receptors, it contains

predominantly GRs (Lupien & Lepage, 2001). Consequently, this suggests a purely physiological explanation as to why there might be no effects of activation of the MRs only on the frontal lobes (i.e., because there are very few receptors available to be over-activated). If the location and abundance of both types of receptors in frontal lobes were similar, different effects might have been found.

The results of Experiment 3 also found that, in participants aged older than 45 years, the longer the duration of treatment with steroids, the greater the detrimental effects on memory performance. These data support the same age by duration of treatment relationship on memory performance identified by Keenan et al. (1995). However, this effect in the older aged patients may be a result of the increase in age itself as opposed to duration of treatment (i.e., an individual's sensitivity to cortisol increases with age; Meaney et al., 1995). Consequently, further studies need to be carried out with younger patients who have had longer durations of treatment to rule out the additional effects of age.

In contrast to Keenan et al., the results of Experiment 3 did not find any evidence of a plateauing effect after the first three years of treatment. However, as Experiment 3 was a retrospective study and none of the participants recruited had been treated with steroids for less than three years, it was not possible to explore this. In addition, as patients with Addison's disease are treated with replacement levels of steroids and not with the same high doses of steroids used to treat other pathologies (e.g., rheumatoid arthritis), the effects on memory produced by replacement doses of steroids would be expected to be different, at least in the shorter term, than those produced by higher than normal doses. Notwithstanding this, the significant

relationship between duration of treatment and detrimental effects on memory performance identified by Experiment 3 does suggest that chronic treatment with replacement levels of cortisol may have detrimental effects on memory, at least, in older patients.

6.3.2. *Summary of the findings*

The results produced by Experiment 3 have extended our existing knowledge concerning the effects of acute changes in cortisol levels on memory brought about via activation of the different corticosteroid receptors in two significant ways.

First, it is the only study to have examined these effects in humans and, whilst the results were not consistent across all memory tasks, they suggest that activation of the MRs only affects declarative memory performance, whereas activation of the GRs affects working memory performance. The same effects were identified in non-primates. The results of Experiment 3 also go some way to support the claim that the MRs and GRs each serve different aspects of information processing. They suggest that a deficiency or inhibition of the MRs impairs selective attention and sensory integration. In contrast, a deficiency of the GRs only affects the consolidation and retrieval aspects of memory. The results also suggest that activation of the GRs is important for the long-term storage of information (De Quervain et al., 1998) and that balanced activation of both receptors is necessary for optimal memory function (De Kloet et al., 1999).

Second, although confounded by the additional potential effects of pathology, the results go some way to suggest that in older adults, chronic

exposure to moderate stress levels of cortisol may have similar effects on memory to those produced by acute exposure to extreme levels of stress. Ideally, further studies now need to be carried out investigating these effects in non-clinical populations to eliminate the potential effects of pathology and also control for baseline levels of cortisol before treatment. The feasibility of such studies, however, may be restricted for ethical reasons.

6.4. Global evaluation

From a global perspective, the results from Experiments 2 and 3 have each made individual contributions towards our understanding of the effects of acute changes in cortisol levels on working and declarative memory. More specifically, whilst the results of Experiment 2 do not suggest that working memory may be more sensitive to acute changes in cortisol levels than declarative memory, they do suggest that declarative memory is more sensitive to chronic changes in cortisol levels. No effects following acute changes in cortisol on any of the aspects of declarative memory were found. This implies that declarative memory may be more vulnerable to the effects of stress over long, as opposed to short, periods of time. Also, if the primate brain is not the major site for the expression of corticosteroids and memory does not equal hippocampus in humans, it seems reasonable to assume that the effects of corticosteroids on declarative memory in humans may only occur following chronic changes or acute changes of extremely high levels of cortisol.

The results of Experiment 2 have also highlighted the importance of considering even the most subtle differences in methodology when determining the effects produced. Moreover, as a result of the more recent findings by De Quervain et al. (2000) and Wolf et al. (2001), it appears that acute cortisol elevation may only

impair material learned before administration. This shows how changes in cortisol levels can affect both learning and memory and highlights the importance of using cognitive tests that are sensitive enough to identify each of these aspects. There also appears to be a need for a difference in cortisol levels between learning and testing for any effects to occur. This timing of change in cortisol levels relative to learning and testing is certainly something that future researchers need to address. Moreover, whilst the results of Experiment 2 suggest that in the short-term there will be no effects on memory performance if the levels of cortisol are the same during learning and testing, it is still unclear whether the same occurs over chronic periods of time. As the additional demands placed on an individual, both physiological and psychological, for coping with acute periods versus chronic periods of stress can be very different, it seems reasonable to assume that the effects of time during learning and testing might be different too. As identified by De Kloet, Vreugdenhil, Oitzl & Joels (1997), it is often neglected that whilst stress hormones can be protective in the short run, they can add to the damage when they are over-produced or not shut off when no longer needed.

Perhaps one of the most significant contributions made by Experiment 2, however, is how even though significant differences in cortisol between the groups and between high- and low-responders within each group were identified, there was no related difference in effects on memory performance. Future research now needs to focus on individual differences in cortisol-response to determine why these differences in response occur. It also needs to identify whether the same effects occur following chronic changes in cortisol levels and/or whether there an optimum point at which individual differences in response fail to have any effect. As the effects of cortisol on memory performance appear analogous to those produced naturally by

ageing, future research could investigate whether the effects of cognitive ageing are different between high- and low-responders. Although no differences were identified in the short-term, any differences in the long-term may help increase our understanding of the ageing process itself. Also, if there is a relationship between level of response and cognitive ageing, there may be something that can be done to change the individual's cortisol response to reduce any harmful effects.

Although not consistent across all memory tasks, the results of Experiment 3 are the first to suggest that corticosteroids can modulate human memory function through differential activation of the MRs and GRs. Specifically that, in the short-term at least, working memory performance appears more sensitive to a deficiency of MRs, whereas episodic memory performance appears more sensitive to a deficiency of the GRs. As increased activation of the GRs occurs during increased periods of stress, this supports the claim that activation of the GRs is important for the storage of long-term information. The results also suggest that balanced activation of both the MRs and GRs is, indeed, necessary for optimal memory performance and that, whilst the location of corticosteroids might be different between rodents and humans, the effects produced by differential activation appear similar.

In addition to their individual contributions, by using the same item-recognition task as that used by Lupien et al. (1999), from a global perspective the results of both studies have shown a consistent lack of effects of cortisol on certain aspects of cognitive function. Specifically, the two studies with very different target-populations (i.e., non-clinical vs. clinical), of different ages (i.e., mean age 20 years vs. 38.3 years), and whose levels of cortisol were manipulated using different methods, showed no effects of changes in cortisol levels on cognitive search strategies.

In terms of the effects of these data on the broader issues relating to stress, cortisol, learning and memory, as recently reported by Lupien & Lepage (2001), "new levels of analysis should be seriously considered by scientists interested in studying the impact of corticosteroids on human cognitive function" (p.152). First, there appears to be a need for 'methodological refinement of the neuroendocrine protocols and to a tighter control of time of cognitive measurements'. For example, as identified by Fehm-Wolfsdorf et al. (1996), the baseline levels of cognitive function are not the same in the morning versus afternoon phase in humans. Such differences in methodology, which have been used to explain discrepancies in results in the past need to be ruled out when comparing results. Second, the recent "analysis of new human brain imaging data shows that memory function cannot be envisioned as a single entity process and each component of learning and memory (encoding, consolidation and retrieval) involves the combined activation of various brain regions" (Lupien & Lepage, 2001; p.152). This highlights the need to use cognitive measures that are sensitive enough to detect each of these components. Moreover, more recent data suggests that, when investigating the effects of stress on memory, one has to take into account that "the brain is not a spectator but rather an active participant in its response to the environment, particularly environmental stress" (p.152). The results of these studies also suggest that cognition in humans is not merely a passive response to a chemical change.

In conclusion to this thesis, therefore, the fact that even only 18 months after Experiments 2 and 3 were carried out significant changes to the original interpretations of the effects of changes in cortisol levels on memory performance can be made, shows that this is still a relatively unknown and exciting area of research.

7. References

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8. Appendices

Checklist of items containing caffeine

Instructions for completion:

Please complete this form from 24 hours prior to each testing session, then bring the completed form along with you to the testing session.

The concentrations of caffeine in coffee and tea depend on the particular bean or leaf, and on how the beverage is prepared.

On average:

Item	Average caffeine content	Average No consumed within past 7 days
A 5oz (150 ml) cup of percolated or drip coffee	120 mg	
A 5oz (150 ml) cup of instant coffee	70 mg	
A 5oz (150 ml) cup of tea	50mg	
A 12oz (360 ml) soft drink, including colas, 'Pepper' drinks and some lemon-lime drinks	30-60 mg	
1oz Chocolate	7 mg	
A 5oz (150 ml) cup of cocoa	7 mg	

Information sheet for participants in the dose-range study

First of all, many thanks for volunteering to take part in this study.

For your information, the purpose of this dose-range study is to ascertain the doses of hydrocortisone which need to be administered to participants taking part in a study looking at the effects of stress hormones on memory. The doses I will ask you to take are 'only marginally greater than the amount of hydrocortisone produced by the human body in a day'. I have a signed declaration by Prof. Stafford Lightman, Head of the Department of Medicine at Bristol University, to confirm this. You can look at this if you want to.

As part of this study I will need you to take a four sets of tablets of hydrocortisone, one each on one of four consecutive Mondays. I will also need you to produce a sample of blood one hour after taking the last tablet in each set. These samples will be obtained by a trained nurse in the Clinical Investigation Unit, Bristol Royal Infirmary. At the same time you will also be asked to provide a saliva sample. Saliva tubes and instructions on how to do this will be given to you at the Clinical Investigation Unit.

The dates and times you will need you to take tablets on are:

At 7am and 8 am on:

- Monday 1st November
- Wednesday 10th November

You will need to arrive at the Clinical Investigation Unit for 9 am to provide a blood sample on these days.

At 2 pm, 3 pm and 4 pm on :

- Monday 15th November
- Monday 29th November

You will need to arrive at the Clinical Investigation Unit for 5 pm to provide a blood sample on these days.

Are you happy to continue? If so, before we go any further, I need to check whether you are:

- Currently steroid-free and have been for at least 6 months.
- Free from: chronic inflammatory disease; psychiatric disorders; obesity; coronary heart disease; sleep disorders; depression; diabetes (or any other 'abnormal' glucose condition) and any other serious medical condition.

- Medication-free (including recreational drugs) for at least 24 hours.

If you have answered YES to each of these questions, then please continue reading.

Each set of tablets has been labelled and packed in a clear bag. Starting with Set 1, then 2, etc., the procedure for taking each set is as follows:

Monday 1st November

You will need to take 20 mg of hydrocortisone (i.e., a total of 2 x 10 mg tablets) as follows:

- At 07.00 hrs, 15 mg hydrocortisone (i.e., 1.5 x 10 mg tablets) with your normal breakfast. **Please do not eat anything after 07.00 hrs,** and

- At 08.00 hrs, 5 mg hydrocortisone (i.e., 0.5 x 10 mg tablet).

When you have taken both tablets, please make your way to the Clinical Investigation Unit for 09.00 hrs to provide blood/saliva samples. (Directions to Clinical Investigation Unit attached.) You will need to ask for Moira Hunt.

Wednesday 10th November

You will need to take 30 mg of hydrocortisone (i.e., a total of 3 x 10 mg tablets) as follows:

- At 07.00 hrs, 20 mg hydrocortisone (i.e., 2 x 10 mg tablets) with your normal breakfast. **Please do not eat anything after 07.00 hrs,** and

- At 08.00 hrs, 10 mg hydrocortisone (i.e., 1 x 10 mg tablet).

When you have taken both tablets, please make your way to the Clinical Investigation Unit for 09.00 hrs to provide blood/saliva samples.

On Monday 15th November

You will need to take 10 mg of hydrocortisone (i.e., a total of 1 x 10 mg tablet) as follows:

- At 14.00 hrs, 5 mg hydrocortisone (i.e., 0.5 x 10 mg tablet), and

- At 15.00 hrs, 2.5 mg hydrocortisone (i.e., 0.25 x 10 mg tablet), and

- At 16.00 hrs, 2.5 mg hydrocortisone (as above).

Please ensure that you eat your normal lunch and breakfast before taking your first tablet at 14.00 hrs. **Please do not eat anything after 14.00 hrs.**

When you have taken all three tablets, please make your way to the Clinical Investigation Unit for 17.00 hrs to provide blood/saliva samples.

On Monday 29th November

You will need to take 15 mg of hydrocortisone (i.e., a total of 1.5 x 10 mg tablet) as follows:

- At 14.00 hrs, 7.5 mg hydrocortisone (i.e., 0.75 x 10 mg tablet). and
- At 15.00 hrs, 5 mg hydrocortisone (i.e., 0.5 x 10 mg tablet), and
- At 16.00 hrs, 2.5 mg hydrocortisone (as above).

Please ensure that you eat your normal lunch and breakfast before taking your first tablet at 14.00 hrs. **Please do not eat anything after 14.00 hrs.**

When you have taken all three tablets, please make your way to the Clinical Investigation Unit for 17.00 hrs to provide blood/saliva samples.

On all four days it is very important that you take each set of tablets at the times specified and in the stated doses. Do you foresee any problems with this? Are you happy to continue with the study?

If the answer is 'No', then I would like to thank you for reading this far. If, however, the answer is 'Yes' I would now like you to sign a Consent form, as your agreement to participate in the study,and then give you the following items:

- One packet containing 8 x 10 mg tablets of hydrocortisone (i.e., a total of 80 mg of hydrocortisone), with a copy of instructions for administration.
- Directions to the Clinical Investigation Unit in the Bristol Royal Infirmary.

Thank you for your co-operation. If you have any problems, please do not hesitate to contact me on either 0117 954 6838 (during office hours) or email address : *M.Y.Tytherleigh@bristol.ac.uk.*

Please also note that you are free to withdraw from this study at any time. An honorarium payment of £20 will be made to you upon completion of the study.

Consent Form

Research into the Effects of Stress Hormones (cortisol) on Memory

Declaration by the participant

I ..... (Full name)

of ..... (Address)

.....

.....

..... (Contact Tel No)

hereby consent to participate in the above study.

- I have had an opportunity to ask questions and discuss the study Yes / No
- I have received satisfactory answers to all my questions Yes / No
- I have received enough information about the study Yes / No

I understand that my involvement in this study is voluntary and that my decision to participate or not to participate will not affect the treatment I receive. I also understand that I am free to withdraw from this study at any time. I understand the purpose of the study and any risks involved. The nature and purpose of all procedures in this study have been explained to me.

Signed ..... Date

.....

Declaration by the investigator

I confirm that I have provided the above named individual with an information sheet and explained the nature of the study and the procedures involved. The participant has given his/her consent freely and voluntarily.

Signed ..... Date .....

Calculation produced for Cohen's effect size

```
>POWER
>MODEL ONEWAY / GROUPS=3 AVGESQ=.75

>Rem POWER
>MODEL ONEWAY / GROUPS=3 AVGESQ=.10

>Rem POWER
>MODEL ONEWAY / GROUPS=3 AVGESQ=.01


>ESTIMATE / ALPHA=0.05 HIGH=20
                                Alpha =        0.050
                  Sample size:  Low =           20
                                High =          20
                            Increment =          1
                                Model =      Oneway
                     Number of groups =           3
                Avg. std. sq. effect =        0.010
           Noncentrality parameter =          0.030 * sample size
                              SAMPLE
                                SIZE          POWER
                             (per cell)
                                  20          0.095
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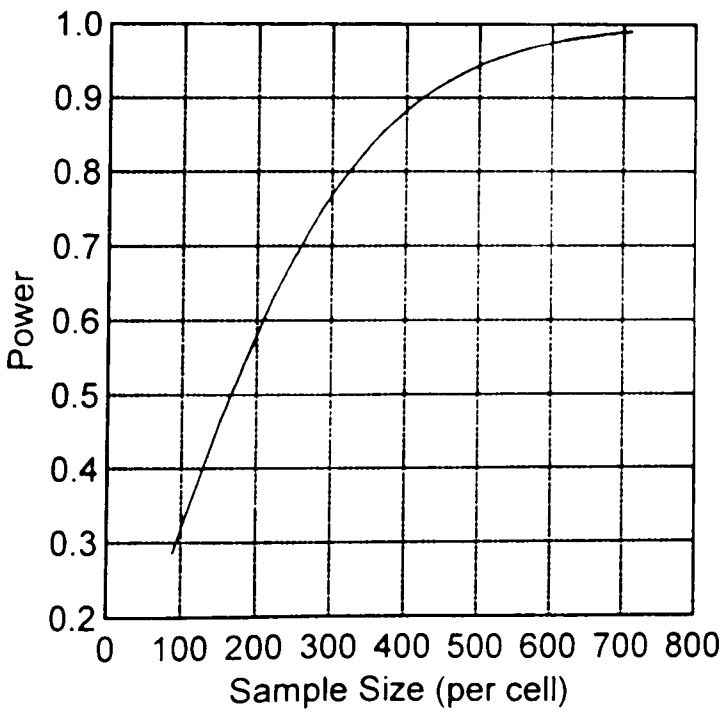

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                                Alpha =        0.050
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                                High  =           20
                            Increment =            1
                                Model =      Oneway
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                Avg. std. sq. effect  =        0.062
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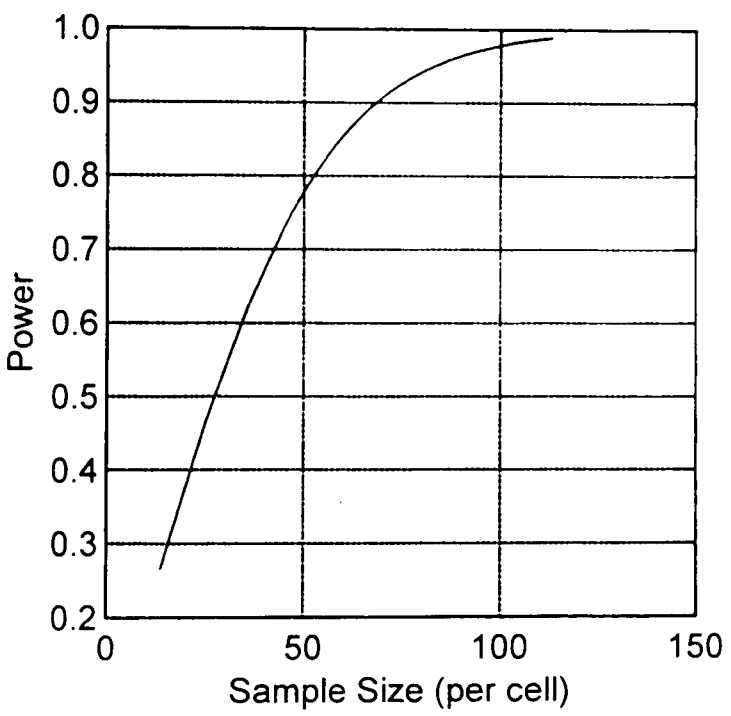


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>ESTIMATE / ALPHA=0.05 HIGH=20
                                Alpha =        0.050
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                                High  =           20
                            Increment =            1
                                Model =      Oneway
                     Number of groups =            3
                Avg. std. sq. effect  =        0.160
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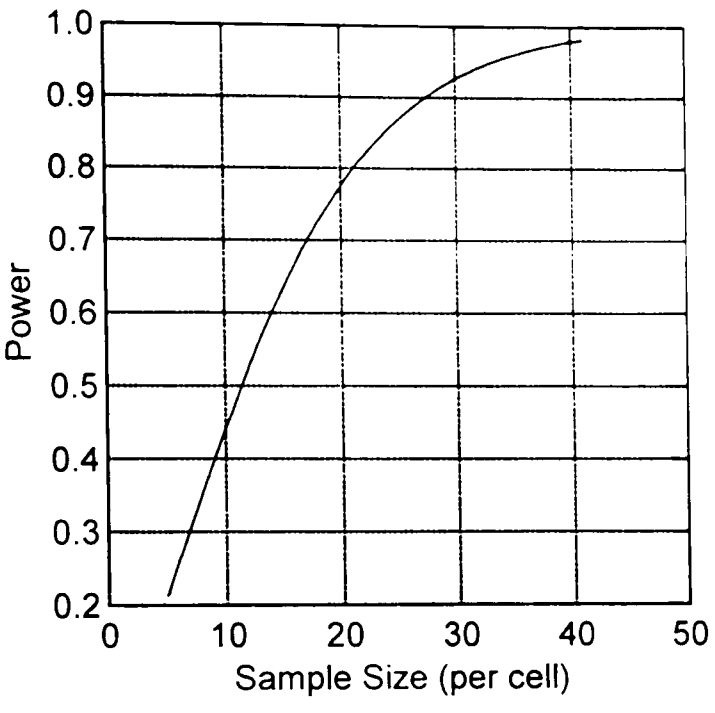
Power Curve (Alpha = 0.050)
Power
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
10
20
30
40
50
Sample Size (per cell)

Information Sheet for Healthy, Young Males – Phase I

Research into the Effects of Stress Hormones (cortisol) on Memory

The aim of this study is to examine how your hormones affect your memory. In particular, we wish to examine how the hormones released when you are stressed affect how well you can remember.

As part of this study you will need to attend three 'testing' sessions. These sessions will take place on three separate days and will be arranged at times that are most convenient for you. Your contribution will be very helpful to this research.

The first of these sessions will be an induction session. As part of this you will be asked to provide some details about yourself and receive some more information about what is expected from you prior to each memory test. You will also receive an opportunity to ask some questions. Your memory will be tested at each of the last two sessions. The format for each session is as follows.

Visit I – Induction

All testing will be held in the Clinical Investigation Unit (CIU), which is located on the fifth floor in the Bristol Royal Infirmary (BRI). The induction session will last approximately one hour.

During this visit you will be asked to:

- Complete questionnaires which measure how you are feeling.
- To provide details of any serious family illnesses.
- Complete a Consent Form.

In addition:

- We will also measure your height and weight.
- We will arrange convenient dates for testing.
- Give you tablets to take on the day of your first memory test
- Give you a check-list of items which contain caffeine.

Visit II - Morning Testing

As part of the morning testing procedure, you will need to do the following:

- Get up in time to take your first tablet by 07.00 hrs, along with your 'normal' breakfast. Please do not eat anything after 07.00 hrs, as we need your sugar levels to have settled two hours prior to memory testing. (Ask the investigator if you would like to receive an early morning wake-up call.)
- Take a second tablet at 08.00 hrs.
- Arrive at the CIU for 08.45 hrs.
- Produce a sample of saliva
- Report how stressed you are feeling, on a scale of 0 (no stress) to 10 (high stress).
- Complete a battery of memory tests at 09.00 hrs.
- Provide a 'finger-prick' sample of blood after testing.
- Tell us what you ate for breakfast.
- If appropriate, make arrangements for the second testing session and collect your second batch of tablets.

Visit III - Afternoon Testing

As above, but with the following exceptions.

- Get up at your usual time and eat your 'normal' meals until 15.00 hrs. Please do not eat anything after 15.00 hrs.
- Take your first tablet at 14.00 hrs.
- Take your second tablet at 15.00 hrs.
- Take your third tablet at 16.00 hrs.
- Arrive at the CIU for 16.45 hrs
- Complete a battery of memory tests at 17.00 hrs.

At the end of both testing sessions, you will be debriefed, as required, and given the opportunity to ask any questions of your own. An honorarium will be paid for your time; under ICH guidelines, this payment will be taxable.

Although we would appreciate your commitment to complete the study, you will be free to withdraw from the experiment at any time. You will also receive our full assurance that confidentiality will be maintained at all times.

If you have any questions or concerns relating to the above, please do not hesitate to contact the researcher, Michelle Tytherleigh, on telephone no : 0117 928 8564.

APPENDIX VI

PERSONAL RECORD SHEET – STUDENT STUDY

ID No :	**Age :**	**Height :**	**Weight :**
BDI Score :			
GHQ Score :			
Details of Family History of Illness (if applicable) :			
High/Low Caffeine user :			**HIGH/LOW**
Consent form received :			**YES/NO**
Dates arranged for testing :			**AM.** **PM.**
Tablets supplied			**YES/NO**
Notes :			

BECK'S DEPRESSION INVENTORY

PARTICIPANT NO		DATE		TIME	

The following questions are about how you feel today, **RIGHT NOW**. Please **read each statement in each section and circle the number of the statement which most closely reflects how you feel right now.** Be sure to read all the statements in each group before you make your choice.

1.	0	=	I do not feel sad
	1	=	I feel sad
	2	=	I am sad all the time and can't snap out of it
	3	=	I am so sad or unhappy that I can't stand it
2.	0	=	I am not particularly discouraged about the future
	1	=	I feel discouraged about the future
	2	=	I feel I have nothing to look forward to
	3	=	I feel the future is hopeless
3.	0	=	I do not feel like a failure
	1	=	I feel I have failed more than the average person
	2	=	As I look back on my life, all I can see is a lot of failures
	3	=	I feel I am a complete failure as a person
4.	0	=	I get as much satisfaction out of things as I used to
	1	=	I don't enjoy things the way I used to
	2	=	I don't get real satisfaction out of anything anymore
	3	=	I am dissatisfied or bored with everything
5.	0	=	I don't feel particularly guilty
	1	=	I feel guilty a good part of the time
	2	=	I feel guilty most of the time
	3	=	I feel guilty all of the time
6.	0	=	I don't feel I am being punished
	1	=	I feel I may be punished
	2	=	I expect to be punished
	3	=	I feel I am being punished
7.	0	=	I don't feel disappointed in myself
	1	=	I am disappointed in myself
	2	=	I am disgusted with myself
	3	=	I hate myself
8.	0	=	I don't feel I am worse than anybody else
	1	=	I am critical of myself for my weaknesses or mistakes
	2	=	I blame myself all the time for my faults
	3	=	I blame myself for everything bad that happens
9.	0	=	I don't have any thoughts of killing myself
	1	=	I have thoughts of killing myself, but I would not carry them out
	2	=	I would like to kill myself
	3	=	I would like to kill myself if I had the chance
10.	0	=	I don't cry anymore than usual

	1	=	I cry more now than I used to
	2	=	I cry all the time now
	3	=	I used to be able to cry, but now I can't cry even though I want to
11.	0	=	I am no more irritated now than I ever am
	1	=	I get irritated or annoyed more easily than I used to
	2	=	I feel irritated all the time now
	3	=	I don't get irritated at all by the things that used to irritate me
12.	0	=	I have not lost interest in other people
	1	=	I am less interested in other people than I used to be
	2	=	I have lost most of my interest in other people
	3	=	I have lost all of my interest in other people
13.	0	=	I make decisions about as well as I ever could
	1	=	I put off making decisions more than I used to
	2	=	I have greater difficulty in making decisions than before
	3	=	I can't make decisions at all anymore
14.	0	=	I don't feel I look any worse than I used to
	1	=	I am worried that I am looking old or unattractive
	2	=	I feel that there are permanent changes in my appearance that make me look unattractive
	3	=	I believe that I look ugly
15.	0	=	I can work about as well as before
	1	=	It takes an extra effort to get started at doing something
	2	=	I have to push myself very hard to do anything
	3	=	I can't do any work at all
16.	0	=	I can sleep as well as usual
	1	=	I don't sleep as well as I used to
	2	=	I wake up 1-2 hours earlier than usual and find it hard to get back to sleep
	3	=	I wake up several hours earlier than I used to and cannot get back to sleep
17.	0	=	I don't get more tired than usual
	1	=	I get tired more easily than I used to
	2	=	I get tired from doing almost anything
	3	=	I am too tired to do anything
18.	0	=	My appetite is no worse than usual
	1	=	My appetite is not as good as it used to be
	2	=	My appetite is much worse now
	3	=	I have no appetite at all anymore
19.	0	=	I haven't lost much weight, if any, lately
	1	=	I have lost more than 5 pounds
	2	=	I have lost more than 10 pounds
	3	=	I have lost more than 15 pounds
19a	I am purposely trying to lose weight by eating less: YES NO (please circle one)		
20.	0	=	I am no more worried about my health than usual
	1	=	I am worried about physical problems such as aches and pains; or upset stomach; or constipation
	2	=	I am very worried about physical problems and it's hard to think of much else

	3	=	I am so worried about my physical problems that I cannot think about anything else
21.	0	=	I have not noticed any recent change in my interest in sex
	1	=	I am less interested in sex than I used to be
	2	=	I am much less interested in sex now
	3	=	I have lost interest in sex completely

GENERAL HEALTH QUESTIONNAIRE

Information Sheet for Healthy, Young Males – Phase II

As you will know by now, the aim of this study is to examine how your hormones affect your memory. In particular, we wish to examine how the hormones released when you are stressed affect how well you can remember. Having already attended the induction session and been recruited as a participant in this study, the details below outline the procedure you will need to follow in preparation for the two 'testing' sessions. It is very important that you follow each procedure as shown, and that you adhere to the times and dosages of tablets you are asked to take. It is also very important that you remain both alcohol and/or medication-free (including recreational drugs) for at least 24 hours prior to testing, and do not consume any food from up to 2 hours prior to testing.

Procedure to follow for Morning Testing only.

Please note that your morning testing session has been arranged for ..... All testing will be held in the Clinical Investigation Unit (CIU), which is located on the fifth floor in the Bristol Royal Infirmary (BRI). Directions to the BRI and the CIU are enclosed.

As part of the morning testing procedure, you will need to do the following:

- Get up in time to take **ALL SIX tablets at 07.00 hrs**, along with your 'normal' breakfast. **Please do not eat anything after 08.00 hrs, as we need your sugar levels to have settled two hours prior to memory testing.** (Please contact the investigator if you would like to receive an early morning wake-up call.) . **Also, please do not drive after taking the tablets.** You will be able to drive after the testing has been completed.
- Arrive at the CIU for **09.50 hrs.**
- Produce a sample of saliva
- Tell us what you ate for breakfast.
- Report how stressed you are feeling, on a scale of 0 (no stress) to 10 (high stress).
- Complete a battery of memory tests **at 10.00 hrs.**
- Provide a 'finger-prick' sample of blood after testing.
- If appropriate, make arrangements for the second testing session and collect your second batch of tablets.

Procedure to follow for the Afternoon Testing only.

Please note that your afternoon testing session has been arranged for .....

As above, but with the following exceptions.

- Get up at your usual time and eat your 'normal' meals until 15.00 hrs. **Please do not eat anything after 15.00 hrs.**
- **Take ALL SIX tablets at 14.00 hrs.** Please do not drive after taking the tablets. You will be able to drive after the testing has been carried out.
- Arrive at the CIU for **16.50 hrs**
- Complete a battery of memory tests **at 17.00 hrs.**

PLEASE DON'T FORGET TO BRING YOUR TICK LIST OF CAFFEINE-CONTAINING ITEMS WHICH SHOULD BE COMPLETED FROM UP TO 24 HOURS PRIOR TO TESTING.

At the end of both testing sessions, you will be debriefed, as required, and given the opportunity to ask any questions of your own. An honorarium will be paid for your time; under ICH guidelines, this payment will be taxable.

Although we would appreciate your commitment to complete the study, you will be free to withdraw from the experiment at any time. You will also receive our full assurance that confidentiality will be maintained at all times.

If you have any questions or concerns relating to the above, please do not hesitate to contact the researcher, Michelle Tytherleigh, on telephone no : 0117 954 6838 (during office hours); 0787 923 5591 (after office hours) or by email :M.Y.Tytherleigh@bristol.ac.uk. Alternatively, if something happens on the day of testing which means that you will not be able to make your appointment, please phone my mobile number to let me know as soon as possible

STUDENT STUDY : PARTICIPANT SCORE SHEETS

ID NO :		**SESSION 1 OR 2**	
TESTING SESSION – AM or PM		**Date :**	
Salivary Cortisol level :			
Blood Glucose level :			
Self-reported stress level :			
Approximate caffeine intake prior to testing :			
Food items consumed during day :			
Memory Test Scores:			
WORKING MEMORY	**Forward Digit Span (1)**		
	Backward Digit Span (1)		
	Backward Digit Span (2)		
	Forward Digit Span (2)		
	Item-recognition Task – errors		
	Item-recognition Task – RT		
	Letter Naming Task:		
	Category Naming Task:		
EPISODIC MEMORY	**Hopkins Verbal Learning : recall**		
	Hopkins Verbal Learning : recog		
	Doors recognition		
	Names recognition		
SEMANTIC MEMORY	**Speed of Comprehension**		
	Spot-the-word		
Notes :			

SAMPLE

Lists for use with Forward and Backward Item Recognition

PARTICIPANT NO : **DATE :** **AM/PM**

Version I

Discontinue after failure on BOTH trials of any items
Administer BOTH trials of each item, even if the subject passes the first trial

Item	DIGITS FORWARD	Mark	DIGITS BACKWARD	Mark
1	1-8		3-6	
2	2-4		7-5	
3	3-6-5		4-8-5	
4	2-4-9		2-6-8	
5	3-1-7-4		5-7-2-4	
6	4-6-2-9		7-6-2-9	
7	1-8-5-2-4		4-7-1-5-9	
8	8-7-1-9-5		2-8-3-6-9	
9	2-4-7-3-9-1		8-3-7-1-4-2	
10	1-9-5-7-4-3		7-8-4-9-3-6	
11	5-6-3-9-2-1-8		8-2-1-9-3-7-4	
12	6-4-3-2-8-5-7		2-7-9-4-9-6-8	
13	2-7-5-8-6-4-9-3		3-1-7-9-4-2-5-8	
14	9-4-3-7-6-2-5-8		7-2-8-1-9-6-5-3	

TOTAL DIGITS FORWARD (1) and (2)	
TOTAL DIGITS BACKWARD (1) and (2)	
OVERALL TOTAL	

SAMPLE

Hopkins Verbal Learning Test

PARTICIPANT NO : **DATE :** **AM/PM**

Version II : Part A : Free Recall List

	Trial 1	Trial 2	Trial 3
TEACHER			
BASKETBALL			
LETTUCE			
DENTIST			
TENNIS			
BEAN			
ENGINEER			
POTATO			
PROFESSOR			
GOLF			
CORN			
SOCCER			

Version II : Part B : Recognition List

TENNIX	Football*	PROFESSOR	Spinach*	Solicitor*	Submarine
GOLF	DENTIST	LETTUCE	Spider	Water	BEAN
BASKETBALL	Doctor*	CORN	Baseball*	TEACHER	Snake
Carrot*	ENGINEER	Glove	SOCCER	POTATO	Tulip

* = related items

Scoring

Each participant's score is calculated as follows:

Free recall no correct	Trial 1	/12	Trial 2	/12	Trial 3	/12
True positives						/12
False positive errors	Related	/6	Unrelated	/6		
Discrimination Index (TP-FP)						

MARKING SCHEDUE FOR THE DOORS TEST

PARTICIPANT NO : **DATE :** **AM/PM**

Put a tick under the response given for the slide

SLIDE NO	NEW	OLD	GUESS	UNSURE	DEFINITE
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					
33					
34					
35					
36					
37					
38					
39					
40					
TOTAL					

MARKING SCHEDUE FOR THE NAMES TEST

PARTICIPANT NO : **DATE :** **AM/PM**

Put a tick under the response given for the slide

SLIDE NO	NEW	OLD	GUESS	UNSURE	DEFINITE
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					
33					
34					
35					
36					
37					
38					
39					
40					
TOTAL					

The Speed and Capacity of Language Processing Test (SCOLP)

The Speed of Comprehension Test

Scoring

Obtain scores from each participant's copy of the test (as attached).

The total number of sentences completed in two minutes should be entered in the summary below. The total number of errors can then be calculated by using the relevant scoring template (refer to instructions provided).

The Spot-the-Word Test

Scoring

The scoring template should be used to mark the number of items correct, after which Table 9 or 10 can be used to obtain a scaled score. These scores should be entered in the relevant boxes on the scoring sheet at the back of the Speed of Comprehension Test Form.

Summary of SCOLP Results

<table>
<tr><th colspan="2">The Speed of Comprehension Test</th><th colspan="2">The Spot-the-Word Test</th></tr>
<tr><td>No completed in 2 minutes</td><td></td><td rowspan="2">No of items correct</td><td rowspan="2"></td></tr>
<tr><td>No of errors</td><td></td></tr>
<tr><td>Scaled score (Table 7)</td><td>B</td><td>Scaled score (Table 9)</td><td>A</td></tr>
<tr><td>Percentile Score (Table 8)</td><td></td><td>Percentile Score (Table 10)</td><td></td></tr>
<tr><td colspan="3">Scaled score discrepancy

Spot-the-Word scaled score (A) minus
Speed of Comprehension scaled score (B)
(see Table 11)</td><td>A-B</td></tr>
</table>

Instructions for how to operate glucose testing kit

Preparing for measurement

- **Wash hands** with soap and warm water; dry thoroughly.
- **Take a test strip out of the vial.**
- **Firmly close the vial immediately** using the correct stopper (the effects of light or humidity can make the test strips unusable).
- **Press the ON/OFF button.**

All the elements of the display screen appear simultaneously for about 2 seconds. Make sure that all the display elements appear in terms of the figure ***8***. If one of the elements is defective, the information shown in the display might be incorrect.

Following the display check, the code number together with the time and date appear and the word **CODE** blinks. Compare the code number displayed with the code number given on the test-strip vial label. The measurement can only be performed if the code numbers are identical.

Measurement of blood glucose

- When the meter is switched on and the **flap closed, insert the test strip into the slot** at the bottom edge of the meter **in the direction of the arrows.** Make sure that the test strip is inserted as far as it will go. When the Accutrend GC has successfully read the test-strip code, two beeps are heard and the word **CODE** stops blinking in the display.
- **Open the flap.** ***12*** **sec** should now blink in the display.
- Prick the side of the finger tip, e.g., with the Softclix Pro lancing device.
- Wipe off the first drop of blood.
- **Apply a large suspended drop of blood to the yellow test pad** on the top of the strip without touching the pad directly with the finger. **The yellow test pad must be completely covered with blood. Do not on any account apply a second drop of blood to the test pad otherwise erroneous results might be obtained.**
- **Close the flap immediately;** wait for display of result.

Display of result and control check

At the end of the reaction time a series of beeps is heard. The result is displayed and automatically stored. If the value obtained in the blood glucose determination is below 1.1 nmol/l (20 mg/dl), the meter displays ***LO.*** If the value obtained in the blood glucose determination is above 33.3 nmol/l (600 mg/dl), the meter displays ***HI.***

To terminate the measurement, proceed as follows:

- Switch of the meter.
- Open the flap.
- Remove the test strip.
- Close the flap.

APPENDIX XII

Details of instructions given to participants, via **Microsoft** Powerpoint, for how to complete the item recognition task.

APPENDIX XIII

Details of instructions given to participants, via Microsoft Powerpoint, for how to complete the Names and the Doors tasks.

Copy of poster displayed throughout departments in University of Bristol as part of recruitment

They say an elephant never forgets,
but what happens when that elephant becomes stressed?

If you are interested in finding out what the effects of stress are on your memory, then we want to hear from you.

We require healthy, young males to take part in a study investigating the effects of stress hormones on memory.

All volunteers must be:

Between 18-25 years.

Either a first year psychology undergraduate or medical undergraduate currently studying at the University of Bristol.

Healthy, both physically and mentally.

Non-smoker

If you are interested and want to find out exactly how good your memory is under stress, please print your name below.
You will also need to provide contact details (i.e., either an email or telephone number) so that we can arrange to discuss the study further.
Alternatively, you can contact me, Michelle Tytherleigh, on M.Y.Tytherleigh@bristol.ac.uk, or Tel : 0117 928 8564

An honorarium will be paid for your time.

Further details relating to the study emailed to volunteers who responded to poster/email

Dear 'potential' volunteer

RE : THE EFFECTS OF STRESS HORMONES ON MEMORY

First of all, many thanks for showing an interest in my study. This promises to be a very interesting study and, hopefully, will produce some very interesting results.

The purpose of this information sheet is to give you some further information about the study and if, after reading this you are still interested in taking part, to arrange an induction meeting. The purpose of this meeting will be to:

- Get you to complete a couple of short questionnaires.
- Obtain details of your age, height and weight.
- Obtain any information relating to any history of family illness.
- Provide you with more information about the study and what you will be asked to do.
- Get you to complete a consent a form
- Arrange two dates for testing – one in the morning and one in the afternoon.
- Give you the opportunity to ask me any questions.

This whole session should only take about 15 minutes to complete.

I am planning to hold these induction meetings throughout the day (i.e., from 9.00 to 5.00) at 30 minute intervals, i.e., 9.00, 9.30, 10.00 etc. Initially, this will take place during the weeks commencing 7th and 14th February. As such, if you could give me some suitable dates and times when you can be available to meet with me during these times, I will get back to you with an appointment time. These meetings will be held in the Department of Experimental Psychology and I will advise you of the room.

I look forward to hearing back from you. In the meantime, if you have any further questions to ask me, please do not hesitate to contact me by email.

Regards

Michelle Tytherleigh
Email : m.y.tytherleigh@bristol.ac.uk

Results of One-way ANOVA's between high- and low-cortisol responders memory scores for each condition and at both times of day

Descriptives

Descriptive Statistics

group condition		N	Minimum	Maximum	Mean	Std. Deviation
stress	SALIVA1	20	82.3	755.2	323.735	179.395
	SALIVA2	20	13.8	235.2	81.435	68.977
	Valid N (listwise)	20				
control	SALIVA1	20	5.2	61.6	20.915	14.314
	SALIVA2	20	1.6	16.0	6.600	3.027
	Valid N (listwise)	20				
blocker	SALIVA1	20	1.7	15.5	4.675	3.789
	SALIVA2	20	.6	5.9	2.340	1.472
	Valid N (listwise)	20				

Oneway

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TOTFOR1	Between Groups	19.142	2	9.571	1.755	.200
	Within Groups	103.631	19	5.454		
	Total	122.773	21			
TOTBAC1	Between Groups	21.051	2	10.525	.603	.557
	Within Groups	331.722	19	17.459		
	Total	352.773	21			
ERROR1	Between Groups	6.633	2	3.317	.910	.419
	Within Groups	69.231	19	3.644		
	Total	75.864	21			
TIME1	Between Groups	4482621	2	2241310	.013	.987
	Within Groups	3.2E+09	19	1.7E+08		
	Total	3.2E+09	21			
LETTER1	Between Groups	99.563	2	49.781	2.847	.083
	Within Groups	332.256	19	17.487		
	Total	431.818	21			
H_RECAL1	Between Groups	7.021	2	3.510	.469	.633
	Within Groups	142.297	19	7.489		
	Total	149.318	21			
H_RECOG1	Between Groups	1.127	2	.564	.596	.561
	Within Groups	17.964	19	.945		
	Total	19.091	21			
NAMES1	Between Groups	2.864	2	1.432	.099	.906
	Within Groups	275.500	19	14.500		
	Total	278.364	21			
DOORS1	Between Groups	12.243	2	6.122	.521	.602
	Within Groups	223.075	19	11.741		
	Total	235.318	21			
SPEED1	Between Groups	27.629	2	13.815	3.379	.056
	Within Groups	77.689	19	4.089		
	Total	105.318	21			
SPOT1	Between Groups	5.091	2	2.545	.579	.570
	Within Groups	83.500	19	4.395		
	Total	88.591	21			
CATEG1	Between Groups	45.694	2	22.847	.509	.609
	Within Groups	852.897	19	44.889		
	Total	898.591	21			

Oneway

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TOTFOR1	Between Groups	4.880	2	2.440	.309	.736
	Within Groups	276.698	35	7.906		
	Total	281.579	37			
TOTBAC1	Between Groups	47.595	2	23.797	1.255	.298
	Within Groups	663.879	35	18.968		
	Total	711.474	37			
ERROR1	Between Groups	20.252	2	10.126	1.506	.236
	Within Groups	235.327	35	6.724		
	Total	255.579	37			
TIME1	Between Groups	6.7E+08	2	3.4E+08	2.307	.115
	Within Groups	5.1E+09	35	1.5E+08		
	Total	5.8E+09	37			
LETTER1	Between Groups	6.499	2	3.250	.163	.851
	Within Groups	699.317	35	19.980		
	Total	705.816	37			
H_RECAL1	Between Groups	34.060	2	17.030	1.083	.350
	Within Groups	550.492	35	15.728		
	Total	584.553	37			
H_RECOG1	Between Groups	9.641E-02	2	4.821E-02	.097	.908
	Within Groups	17.377	35	.496		
	Total	17.474	37			
NAMES1	Between Groups	62.722	2	31.361	2.733	.079
	Within Groups	401.620	35	11.475		
	Total	464.342	37			
DOORS1	Between Groups	20.160	2	10.080	.804	.456
	Within Groups	438.892	35	12.540		
	Total	459.053	37			
SPEED1	Between Groups	4.196	2	2.098	.246	.784
	Within Groups	299.067	35	8.545		
	Total	303.263	37			
SPOT1	Between Groups	1.730	2	.865	.187	.831
	Within Groups	162.165	35	4.633		
	Total	163.895	37			
CATEG1	Between Groups	.573	2	.286	.006	.994
	Within Groups	1720.795	35	49.166		
	Total	1721.368	37			

Oneway

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TOTFOR2	Between Groups	1.126	2	.563	.057	.945
	Within Groups	198.179	20	9.909		
	Total	199.304	22			
TOTBACK2	Between Groups	37.628	2	18.814	.997	.387
	Within Groups	377.589	20	18.879		
	Total	415.217	22			
ERRORS2	Between Groups	.304	2	.152	.016	.984
	Within Groups	189.000	20	9.450		
	Total	189.304	22			
TIME2	Between Groups	3.9E+08	2	2.0E+08	.869	.435
	Within Groups	4.5E+09	20	2.3E+08		
	Total	4.9E+09	22			
LETTERS2	Between Groups	23.362	2	11.681	.583	.567
	Within Groups	400.464	20	20.023		
	Total	423.826	22			
HOP2RECA	Between Groups	8.413	2	4.207	.248	.783
	Within Groups	339.500	20	16.975		
	Total	347.913	22			
HOP2RECO	Between Groups	.474	2	.237	.660	.528
	Within Groups	7.179	20	.359		
	Total	7.652	22			
NAMES2	Between Groups	1.324	2	.662	.052	.950
	Within Groups	256.589	20	12.829		
	Total	257.913	22			
DOORS2	Between Groups	1.006	2	.503	.026	.974
	Within Groups	385.429	20	19.271		
	Total	386.435	22			
SPEED2	Between Groups	5.175	2	2.587	.287	.754
	Within Groups	180.304	20	9.015		
	Total	185.478	22			
SPOT2	Between Groups	1.936	2	.968	.186	.831
	Within Groups	103.804	20	5.190		
	Total	105.739	22			
CAT2	Between Groups	134.609	2	67.304	1.785	.193
	Within Groups	754.000	20	37.700		
	Total	888.609	22			

Oneway

ANOVA

		Sum of Squares	df	Mean Square	F	Sig
TOTFOR2	Between Groups	.823	2	.412	.076	.92[illegible]
	Within Groups	183.609	34	5.400		
	Total	184.432	36			
TOTBACK2	Between Groups	21.888	2	10.944	.845	.438
	Within Groups	440.436	34	12.954		
	Total	462.324	36			
ERRORS2	Between Groups	22.080	2	11.040	.852	.435
	Within Groups	440.353	34	12.952		
	Total	462.432	36			
TIME2	Between Groups	1.4E+08	2	7.1E+07	.438	.649
	Within Groups	5.5E+09	34	1.6E+08		
	Total	5.7E+09	36			
LETTERS2	Between Groups	57.037	2	28.519	1.010	.375
	Within Groups	959.936	34	28.233		
	Total	1016.973	36			
HOP2RECA	Between Groups	22.337	2	11.169	.793	.461
	Within Groups	478.744	34	14.081		
	Total	501.081	36			
HOP2RECO	Between Groups	1.044	2	.522	1.359	.271
	Within Groups	13.064	34	.384		
	Total	14.108	36			
NAMES2	Between Groups	13.813	2	6.907	1.039	.365
	Within Groups	225.917	34	6.645		
	Total	239.730	36			
DOORS2	Between Groups	15.639	2	7.819	.611	.549
	Within Groups	435.064	34	12.796		
	Total	450.703	36			
SPEED2	Between Groups	34.042	2	17.021	3.602	.038
	Within Groups	160.660	34	4.725		
	Total	194.703	36			
SPOT2	Between Groups	6.482	2	3.241	.814	.452
	Within Groups	135.410	34	3.983		
	Total	141.892	36			
CAT2	Between Groups	86.235	2	43.117	1.837	.175
	Within Groups	798.090	34	23.473		
	Total	884.324	36			

APPENDIX XVII

Letter sent out to Addison's patients asking for volunteers for study

Research into the Effects of Steroids on Memory

Dear

I am writing today to ask if you would be willing to take part in a study looking at how different steroids can affect how much you remember.

This study is being supervised by myself and forms part of the PHD Research being carried out by Michelle Tytherleigh, at The Department of Experimental Psychology, University of Bristol. As one of very few people with Addison's Disease, your contribution will be very helpful to this research.

The overall purpose of this study is to examine how the hormones released when you are stressed affect how well you can remember things. These same effects are produced by steroids. In particular, we are interested in whether different types of steroids have different effects on your memory.

If you would like to discuss the study in more detail, please return the reply slip below. An S.A.E. is enclosed. Upon receipt of this Michelle will make contact with you to discuss the study further.

Many thanks in anticipation of your assistance.

Yours sincerely

Stafford Lightman
Professor of Medicine and Head of Department

Enc : S.A.E.

Research into the Effects of Steroids on Memory

Please complete and return this slip, using the S.A.E. provided, if you wish to discuss the study further.

..... (print name)

..... (address)

..... (address)

..... (contact telephone number)

..... (signature)

Please indicate by selecting YES or NO if you would be happy to discuss the study as a group.YES/NO

APPENDIX XVIII

Letter sent out to Addison's patients with details of initial meeting to discuss study

«Title» «Christian» «Surname»
«Address_1»
«Address_2»
«Address_3»
«Address_4»

17th February 2000

Dear «Title» «Surname»

Re : Meeting to discuss study looking at the effects of steroids on memory

This is to confirm that the above meeting will be held on Wednesday 23rd February at 18.00 hrs. It is anticipated that it will last no longer than one hour.

The venue for the meeting will be the MSc Seminar Room, which is located on the fifth floor in the Bristol Royal Infirmary (BRI). To find this, you will need to enter the BRI from the front entrance at 8 Marlborough Street and take the lift to the fifth floor. This will bring you out onto Ward 28. On exiting the lift, you will then need to turn right into the ward and continue along the corridor until you come to the service elevator. At this point, on your right hand side, you will see a notice directing you to the seminar room.

Both Prof. Lightman and myself will be present at the meeting, the purpose of which is to provide you with more details about the study and answer any queries you may have. If, after hearing this information, you wish to take part, I will then need to advise your GP accordingly. As such, I would appreciate it if you could bring your GP's details along with you.

Please do not hesitate to contact me if you have any further queries, otherwise I will look forward to meeting you on 23rd.

Kind Regards.

Yours sincerely

Michelle Tytherleigh
Department of Experimental Psychology

Information Sheet given to Addison's patients at initial information meeting

This information sheet confirms details of the study looking at the effects of steroids on memory which were presented to you at tonight's meeting.

As explained to you, as part of this study you will need to attend three 'testing' sessions. These will be held on three separate days, with a period of one month between each session. The dates for these sessions will be arranged at times convenient to you.

Female Participants only : Female participants will only be tested between days 5 and 12 of their menstrual cycle.

48 Hours Prior to Each Testing Session

In addition to attending a testing session, we would also like you to change the types of steroids you are taking for 48 hours prior to each testing session. Professor Lightman will organise this for you and, if you have any further concerns, we will be happy to discuss these with you beforehand. You will only need to replace your normal steroids for 48 hours. These will be different types on each occasion, but once each testing session has been carried out, you will be able to revert back to your original treatment.

The format for each testing session will be the same each time. It is only the types of steroids that will alter.

Procedure for each Testing Session

All testing will be carried out in the Clinical Investigation Unit (CIU), which is located on the fifth floor in the
Bristol Royal Infirmary (BRI). Each testing session will last approximately two hours.

As part of the testing procedure, you will need to:

- Complete and return a Consent Form.
- Get up in time to have eaten your normal breakfast by 08.00 hrs. Please do not eat anything after 08.00 hrs, as we need your sugar levels to have settled two hours prior to memory testing.
- Be available at the CIU for 10.45 hrs.
- Tell us what you ate for breakfast.
- Report on how stressed you feel, on a rating scale from 0 (no stress) to 10 (high stress).
- Complete a battery of memory tests at 11.00 hrs.
- Provide a sample of blood to assess your steroid and glucose levels.
- If appropriate, make arrangements for the next testing session and collect your next batch of tablets.

Additional Requirements for the First Testing Session Only

On your first testing day you will be asked to:

- Complete questionnaires which measure how you are feeling.
- Complete a questionnaire to measure your IQ.

We will also measure your height and weight.

Upon completion of all three testing sessions, you will be given more information about the purpose of the study and an opportunity to ask any questions of your own.

We really appreciate your participation in this study. Although we would like your commitment to complete the study, please note that you will be free to withdraw from the experiment at any time. You will also receive our full assurance that confidentiality will be maintained at all times.

If you have any questions or concerns relating to the above, please do not hesitate to contact the researcher, Michelle Tytherleigh, on telephone no : 0117 928 8564.

STUDY LOOKING AT THE EFFECT OF STEROIDS ON MEMORY

Now that you have had the opportunity to hear more about the study, ask some questions and find out what you will be required to do, I now need to know if you would like to take part.

If you do not want to take part, at this point I would like to thank you very much for attending this meeting and assure you that your details will be removed from my database. If, however, you would like to participate, I will need to obtain a few further details and ask you to sign a consent form. Please complete the information below and return it using the S.A.E. provided:-

NAME : .....

DATE OF BIRTH : .....

BRI HOSPITAL NO : .....

GP'S NAME AND ADDRESS : .....

.....

.....

.....

As mentioned in the information sheet, all testing will need to be carried out at 11.00 hrs on each day of testing. As such, I need to know which days would be most convenient for this testing to take place. As many of you work during the week, I will be testing at weekends. Please indicate the most suitable days below, noting that there will be at least one month between each of the 3 individual testing sessions.

.....

.....

Once I have gathered all the information I need and your GP has been informed of the study, I will contact you to arrange testing dates. In the meantime, please do not hesitate to contact me if you require any further information (Tel : 0117 954 6847 with answerphone).

Regards,

Michelle Tytherleigh

Females only.
The testing of females will need to be carried out during days 5 and 12 of the cycle. If you know the approximate dates for this, please write these below.

.....

APPENDIX XX

Letter sent out to each Addisons patients' GP

Dr *[Doctor's name]*
Address 1
Address 2
Address 3

Date

Dear Dr *[Doctor's name]*

Re : **Research study into the effects of steroids on memory**
Your patient: *[patient's name]*
Of : *[patient's address]*

Your patient, [*patient's name*], has kindly volunteered to take part in a study designed to clarify whether steroid hormones – and in particular the glucocorticoid Dexamethasone and the mineralocorticoid Fludrocortisone - are important in the processes underlying memory. The reason we want to perform a study in patients with Addison's disease is that their lack of endogenous steroids allows us to replace them, for a short time only, with either mineralocorticoids alone or glucocorticoids alone.

The study itself is very simple. There will be three periods of testing and on each occasion your patient will – for 60 hours only – replace their normal steroid medication with either:

1. Dexamethasone 1mg O.D.,
2. Fludrocortisone 0.2 mg O.D., or
3. A combination of Dexamethasone 1mg and Fludrocortisone 0.2 mg.

They will then have some simple psychological tests of memory function, have one blood sample taken and revert immediately to their normal steroid replacement regime. There is no reason to expect that this short change in their steroid replacement will have deleterious effects, although it is possible that on the Fludrocortisone alone, they might feel a little more tired – so they should avoid extremely heavy physical exercises during these times of change in steroid replacement.

If you feel there is any further information I should know about your patient, or if you feel there is any reason why your patient should not take part in this study, I should be grateful if you could let me know.

With kind regards

Yours sincerely

Stafford Lightman

SAMPLE LETTER

Letter sent to volunteers with selection of dates for testing

«Title» «christian» «surname»
«Address_1»
«Address_2»
«Address_3»
«Address_4»

Date

Dear «christian»

STUDY LOOKING AT THE EFFECTS OF STEROIDS ON MEMORY

Following on from our recent telephone conversation, I am now pleased to confirm that I have received the medication for my study and am now in a position to arrange the first testing date.

I note from the information attached to your consent form that you can be available for testing on «dates». As such, I should be grateful if you would complete the attached form, indicating which of the dates in June you could be available for testing, and then return it to me using the SAE provided. Upon receipt of this, I will then select one of these dates, contact you to confirm it and then make the necessary arrangements to send your medication out to you. Full details of how to administer the medication will be included.

Please do not hesitate to contact me if you require any further information.

I look forward to receiving your response.

Yours sincerely

MICHELLE TYTHERLEIGH

Office : 0117 954 6847; Mobile : 0403 353033 (with answerphone)

Enc : Response form for completion and return
SAE

RESPONSE FORM TO BE RETURNED USING S.A.E. PROVIDED

INSTRUCTIONS FOR COMPLETION

*Please put a tick next to the dates which are most appropriate to you. I will then select **one** date for testing.*

From :

I can be available for testing on the following dates:

Date	✓ if approp	**Date**	✓ if approp	**Date**	✓ if approp
Friday 2nd June		Monday 5th June		Tuesday 6th June	
Thursday 8th June		Friday 9th June		Monday 12th June	
Tuesday 13th June		Thursday 15th June		Friday 16th June	
Monday 19th June		Tuesday 20th June		Thursday 22nd June	
Friday 23rd June					

ADDITIONAL INFORMATION

To help you make your decision, please note that you will need to be at the Bristol Royal Infirmary for 10.45 hrs on each day of testing. The full testing procedure will only last approximately one hour.

This form is to be used for the first testing session only. We will arrange the second and third testing sessions at Testing Session 1.

SAMPLE

Registered letter sent to volunteers with confirmed date of first testing session, tablets and instructions for administration

«Title» «christian» «surname»
«Address_1»
«Address_2»
«Address_3»
«Address_4»

Date

Dear «christian»

STUDY LOOKING AT THE EFFECTS OF STEROIDS ON MEMORY

Please find enclosed the six tablets and information sheets for the first testing session.

If you require any further information, please do not hesitate to contact me; I can be contacted during office hours on 0117 954 6847, or at home on 0117 974 1810 (with answerphone) at any other time.

If I don't hear from you beforehand, I will look forward to seeing you @ 10.45 hrs on <date> in the Clinical Investigation Unit.

Yours sincerely

MICHELLE TYTHERLEIGH

Enc : 6 tablets
Copy of testing procedure
Caffeine intake tick list

Study looking at the effects of Steroids on memory

Tick List of Items Containing Caffeine
(as produced by N.L. Benowitz, M.D., 1990)

Testing Date :

Dear Participant .....

As part of my study, I thought I might investigate whether the levels of caffeine consumed 24 hours prior to testing have any significant effects upon the affects of stress hormones on memory performance. As such, I would like you to complete this tick list 24 hours prior to each testing session. This means that:

- if you are being testing at 09.00 hrs, please start recording the number of items you have consumed which contain caffeine from 09.00 hrs on the previous day.

- if you are being tested at 17.00 hrs, please start recording the number of items you have consumed which contain caffeine from 17.00 hrs on the previous day.

The concentrations of caffeine in coffee and tea depend on the particular bean or leaf, and on how the beverage is prepared. However, please treat the following as average:

Item	**Average caffeine content**	**Average No of cups consumed within past 24 hours**
A standard (150 ml) cup of percolated or drip coffee	120 mg	
A standard (150 ml) cup of instant coffee	70 mg	
A standard (150 ml) cup of tea	50mg	
A 12oz (360 ml) soft drink, including colas, 'Pepper' drinks and some lemon-lime drinks	30-60 mg	
1oz Chocolate	7 mg	
A 5oz (150 ml) cup of cocoa	7 mg	
One can energy drink, e.g., Red Bull	75 mg	
Other items containing caffeine but not listed (please enter details and quantities consumed)		

Please bring this tick list with you to your testing session.

Study looking at the effects of Steroids on memory

Procedure to follow during testing

As you will aware from your meeting with myself and Prof. Lightman, the purpose of this study is to examine the effects of steroids on memory performance. As a participant, you have been asked to attend three testing sessions, each commencing at 11.00 hrs over three separate days. Following on from our earlier correspondence, I am pleased to confrim that the date for your first testing session is .....

48 Hours Prior to Each Memory Testing Session

In addition to attending a memory testing session, we need you to change the types of steroids you are taking for a total period of two and a half days, i.e., 60 hours. In your case, this means discontinuing your normal medication on the evening of ..... and replacing it with the 'test' steroids on the morning of .....

We would like you to take **2 test tablets only on each** of the three mornings leading up to testing (i.e., a total of 6 tablets, for the three days, is enclosed with this sheet). In addition, if possible we would like you to take these 2 tablets between 07.00 and 08.00 hrs on each of the three mornings. You will not have to take any medication in the afternoon during the first 48 hours. This is because the medication we want you to take has a long half-life in plasma.

In addition, on the day of memory testing only, i.e., on ..... we would also like you to have eaten your normal breakfast before 09.00 hrs. PLEASE DO NOT EAT OR DRINK ANYTHING AFTER 09.00 HRS AS WE WOULD LIKE TO ENSURE THAT YOUR SUGAR LEVELS HAVE SETTLED BEFORE TESTING. If, however, there are medical reasons why you should eat during this two hour period, e.g., diabetes, please do so. Once testing has been carried out, you will then revert to your normal medication, i.e., on the afternoon of ..... (day 3).

The format for each testing session will be the same each time. It is only the types of steroids that will alter. As you will be aware, for the purpose of this study we cannot tell you which tablets you will be asked to take on each occasion. However, these details will be supplied to your GP if required. We have already written to your GP's to inform them of your participation in this study.

Procedure for each Testing Session

All testing will be carried out in the Clinical Investigation Unit (CIU), which is located on the fifth floor in the Bristol Royal Infirmary (BRI). Each testing session will last approximately one hour.

As part of the testing procedure, you will need to:

24 hours prior to memory testing

- Remain alcohol- and/or recreational drug-free for 24 hours prior to memory testing.
- Record your approximate caffeine intake (using the tick sheet enclosed) for 24 hours prior to memory testing. Please bring this tick sheet with you to the memory testing session.

On the day of memory testing

- Get up in time to take your final 2 tablets of medication by 08.00 hrs and have eaten your normal breakfast by 09.00 hrs. **Please do not eat anything after 09.00 hrs, as we need your sugar levels to have settled two hours prior to memory testing.**
- Be available at the CIU for 10.45 hrs.
- Tell us what you ate for breakfast.
- Report on how stressed you feel, on a rating scale from 0 (no stress) to 10 (high stress).
- Complete a battery of memory tests at 11.00 hrs. This should last approximately 40 minutes.
- Provide an intravenous sample of blood to assess your steroid and glucose levels. This will be obtained by a qualified nurse in the BRI.
- If appropriate, make arrangements for the next testing session and collect your next batch of tablets.

Additional Requirements for the First Testing Session Only

On your first testing day only you will be asked to:

- Complete questionnaires which measure how you are feeling.
- Complete a questionnaire to measure your IQ.

We will also measure your height and weight.

Upon completion of all three testing sessions, you will be given more information about the purpose of the study and an opportunity to ask any questions of your own.

We really appreciate your participation in this study. Although we would like your commitment to complete the study, please note that you will be free to withdraw from the experiment at any time. You will also receive our full assurance that confidentiality will be maintained at all times.

If you have any questions or concerns relating to the above, please do not hesitate to contact the researcher, Michelle Tytherleigh, on telephone no : 0117 954 6847 (during office hours) or home no : 0117 974 1810 (with answerphone) at other times.

Instructions for subsequent testing sessions

Study looking at the effects of Steroids on memory

Procedure to follow during testing

I am pleased to confirm that the date of your next testing session is ..... In preparation for this, I need you to follow the procedure below.

48 Hours Prior to Each Memory Testing Session

In addition to attending a memory testing session, we need you to change the types of steroids you are taking for a total period of two and a half days, i.e., 60 hours. In your case, this means discontinuing your normal medication on the evening of .....and replacing it with the 'test' steroids on the morning of .....

We would like you to take **2 test tablets only on each** of the three mornings leading up to testing (i.e., a total of 6 tablets, for the three days, is enclosed with this sheet). In addition, if possible we would like you to take these 2 tablets between 07.00 and 08.00 hrs on each of the three mornings. You will not have to take any medication in the afternoon during the first 48 hours. This is because the medication we want you to take has a long half-life in plasma.

In addition, on the day of memory testing only, i.e., on , we would also like you to have eaten your normal breakfast before 09.00 hrs. PLEASE DO NOT EAT OR DRINK ANYTHING AFTER 09.00 HRS AS WE WOULD LIKE TO ENSURE THAT YOUR SUGAR LEVELS HAVE SETTLED BEFORE TESTING. If, however, there are medical reasons why you should eat during this two hour period, e.g., diabetes, please do so. Once testing has been carried out, you will then revert to your normal medication, i.e., on the afternoon of (day 3).

The format for the testing session is the same as last time; only the types of steroids you have been given have changed. As before, for the purpose of this study we cannot tell you which tablets you have been given to take, but these details will be supplied to your GP if required.

Procedure for each Testing Session

As before, the testing will be carried out in the Clinical Investigation Unit (CIU) and this will last approximately one hour. As part of the testing procedure, you will need to:

24 hours prior to memory testing

- Remain alcohol- and/or recreational drug-free for 24 hours prior to memory testing.
- Record your approximate caffeine intake (using the tick sheet enclosed) for 24 hours prior to memory testing. Please bring this tick sheet with you to the memory testing session.

On the day of memory testing

- Get up in time to take your final 2 tablets of medication by 08.00 hrs and have eaten your normal breakfast by 09.00 hrs. **Please do not eat anything after 09.00 hrs, as we need your sugar levels to have settled two hours prior to memory testing.**
- Be available at the CIU for 10.45 hrs.
- Tell us what you ate for breakfast.
- Report on how stressed you feel, on a rating scale from 0 (no stress) to 10 (high stress).
- Complete a battery of memory tests at 11.00 hrs. This should last approximately 40 minutes.
- Provide an intravenous sample of blood to assess your steroid and glucose levels. This will be obtained by a qualified nurse in the BRI.
- If appropriate, make arrangements for the next testing session and collect your next batch of tablets.

Upon completion of all three testing sessions, you will be given more information about the purpose of the study and an opportunity to ask any questions of your own.

We really appreciate your participation in this study. Although we would like your commitment to complete the study, please note that you will be free to withdraw from the experiment at any time. You will also receive our full assurance that confidentiality will be maintained at all times.

If you have any questions or concerns relating to the above, please do not hesitate to contact the researcher, Michelle Tytherleigh, on telephone no : 0117 954 6847 (during office hours) or home no : 0117 974 1810 (with answerphone) at other times.

Addison's patients' record sheets

ID No:	Age:	Height:	Weight:
BDI Score:			
GHQ Score:			
NART :			
Details of Family History of Illness (if applicable): **Normal medication regime:** **Other medication:** **Other pathologies:**			

High/Low caffeine user	**HIGH/LOW**
Consent form received	YES/NO
Dates arranged for testing	AM. PM.
Tablets supplied	YES/NO
Notes :	

National Adult Reading Test (NART)
word list and instructions for administration and interpretation.

Debriefing letter sent out to Addison's patients

«Title» «christian» «surname»
«Address_1»
«Address_2»
«Address_3»
«Address_4»

Date

Dear «christian»

RE : RESEARCH INTO THE EFFECTS OF STEROIDS ON MEMORY

First of all, I hope this letter finds you fit and well.

As promised, I am now enclosing a summary of your scores for each of the different memory tasks I asked you to complete in each of the three testing sessions. They may not mean much to you as they stand, but you might be interested to see how well your memory stood up to each of the different steroid conditions.

As I mentioned at the end of the last testing session, I would also like to give you a bit of background information about some of the previous research which has been carried out looking at the effects of cortisol on memory. Unfortunately there doesn't appear to be anything which refers directly to people coping with Addison's disease, and this is one reason why this research is so important. I've tried to give you this information in a clear and simple way, and hope you will not be put off by the medical jargon I have had to use.

As I mentioned to you at the start of the testing sessions, the purpose of this study is to look at the effects of steroids on memory. Previous research has shown that high levels of cortisol, such as that produced either by stress or by taking steroids, can be detrimental to memory performance. However, the effects produced are reversible if the treatment is stopped and have been explained as being a result of the over-activation of the cortisol receptors which are located throughout the brain.

Basically, there are two types of cortisol receptors. These are mineralocorticoids, which are activated at normal, basal, levels of cortisol, and glucocorticoids. These latter receptors are activated at stress levels of cortisol. The evidence to date suggests that it is the over-activation of the glucocorticoids which can be 'detrimental' to memory performance.

The mineralocorticoids and glucocorticoids are located, predominantly, in the frontal and hippocampal regions of the brain. These are the two areas which are responsible for the storage of short-term memory (in the frontal lobes) and long-term 'declarative' memory (in the hippocampus). (The term 'declarative' refers to the long-term memory for facts. This is in contrast to 'procedural' memory, which is the long-term memory for procedures e.g., learning to bake a cake or ride a bike. Procedural memory is not associated with the hippocampus and, consequently, is not affected by high levels of cortisol.

The memory tasks I asked you to complete were designed to measure short-term memory and long-term declarative memory only. Basically, the digit-span tasks and the reaction time task are designed to measure your short-term memory, whilst the other tasks are for long-term memory.

So why use people with Addison's. Well, as you know, a person with Addison's Disease does not produce significant levels of cortisol. Consequently, by giving you different types of steroids (i.e., either Fludrocortisone only, Dexamethasone only, or a combination of the two), we were able to control which of the two types of cortisol receptors were activated. By measuring your memory performance under each of the different conditions, we can now analyse the results to see whether the activation of one type of receptor is more 'harmful' to memory than the other/s. For your further information, Fludrocortisone activates the mineralocorticoids only, Dexamethasone activates the glucocorticoids only, and a combination of the two activates both types of receptors. By giving you a combination of fludrocortisone and hydrocortisone as part of your normal regime, both types of cortisol receptors are activated.

I am sure you will understand that, at this point , I cannot tell you what the results of this study are. To do this I need to finish collecting all the data (which I hope will be done by the end of November) and then analyse the results. However, as soon as I have done this, I will let you know what we find.

If you would like to discuss any of this information with me further, please do not hesitate to contact me during office hours on 0117 928 8556. In the meantime, I hope this bit of background information has been helpful. I cannot thank you enough for all your help in this study and wish you continued good health and happiness in the future.

Kind Regards

Yours sincerely

MICHELLE TYTHERLEIGH

Enc : Copy of your score sheet

SCORES FOR ..... AS PART OF STUDY LOOKING AT EFFECTS OF STEROIDS ON MEMORY PERFORMANCE

SHORT TERM MEMORY TASKS				LONG-TERM SEMANTIC MEMORY		LONG-TERM EPISODIC MEMORY				LONG-TERM SEMANTIC MEMORY						
TOTAL Forward Digit Span (out of 28)	TOTAL Backward Digit Span (out of 28)	Reaction Time task - total errors (out of 108)	Reaction time - total in secs	Letter Naming task	Letter given	TOTAL no of items recalled (out of 36)	TOTAL no of items recognised (out of 12)	TOTAL Names Recalled (out of 40)	TOTAL Doors Recalled (out of 40)	Scaled score for Silly Sentences (out of 18)	Category Naming task	Category given	Glucose Levels	Caffeine Levels	Self-stress Score	Condition and session no
18	15	2	97266	14	A	22	12	32	32	10	29	Body	4.7	1167	8	Flud only
18	11	8	59698	16	F	24	12	35	33	12	24	Animals	4.7	527	4	DEX only
15	15	5	117727	23	S	33	12	33	34	10	20	Fruit	4.4	2246	4.4	Combo Flud/DEX

Other Information

IQ calculated score using NART : 106
Body Mass Index : 39
Beck's depression inventory score : 12 (out of a possible 63, so this is low)
General Health Questionnaire score : 59 (out of a possible 120, so this is low)

Calculation of Power effect sizes produced for Addison's study using MOT2-1

	Working memory	Episodic memory	Semantic memory
Test significance level	0.05	0.05	0.05
Number of levels, M	3	3	3
• Variance of means	66.667	66.667	66.667
• SD at each level	25.000	7.500	3.250
• Between levels correlation	0.700	0.500	0.850
Effect size	0.3556	0.5926	1.6831
Power (%)	71	91	99
N	9	9	9

Actual Means and SD's

Task	Condition					
	MR's only		GR's only		MR's/GR's	
	Mean	SD	Mean	SD	Mean	SD
Working memory[1]	86.44	26.28	85.11	26.56	87.78	21.41
Episodic memory[2]	106.00	10.84	109.89	7.51	112.33	5.72
Semantic memory	13.89	3.22	14.33	3.28	14.44	3.32

Predictions made were that people would perform best under MR's/GR's condition, worse under GR's only condition, and MR's would be somewhere in between.

[1] Comprising scores for Total Digits Forward, Total Digits Backward, COWA and Category Naming Task.
[2] Comprising scores for Hopkins Verbal Learning Test, the Names Recognition Task and the Doors Recognition Task.

Estimated Means

Task	Condition					
	MR's only		GR's only		MR's/GR's	
	Mean		Mean		Mean	
Working memory	80		70		90	
Episodic memory	115		110		120	
Semantic memory	14		12		16	

Carried out power analyses to see how significant my results were in relation to the sample size.

For working memory, with a sample of N=9 participants, and three conditions, the power was 0.71. This was high because of the high between level correlations.

For episodic memory, with a sample of N=9 participants, and three conditions, the power was 0.91. Although the between level correlations were not as high for this, they were quite high .

For working memory, with a sample of N=9 participants, and three conditions, the power was > 0.99. This was high because of the high between level correlations. Also, there may be a smaller effect of condition on this type of task.

Results of series of Pearson's Product Moment Correlations and Spearman's rho correlations between participant's characteristics and total scores for each of the different aspects of memory irrespective of condition

Correlations

		AGE	TREATDUR	IQ	BDI	GHQ	BMI	TOTDIGFO	TOTDIGBA	TOTLETTE	TOTCAT	TOTERR	TOTRT	TOTHRECA	TOTHRECO	TOTDOOR	TOTNAME	TOTSPEED	LOGSPOT
AGE	Pearson Correlation	1.000	.382	.243	-.386	-.297	.111	.159	-.055	.545	.612	.068	.216	.164	.061	.221	.220	.220	.282
	Sig. (2-tailed)	.	.310	.530	.305	.438	.776	.683	.889	.129	.080	.872	.577	.674	.875	.568	.569	.569	.463
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TREATDUR	Pearson Correlation	.382	1.000	-.283	-.422	-.571	-.208	.139	.160	.410	.391	-.485	-.272	.332	-.073	.252	-.158	.193	.581
	Sig. (2-tailed)	.310	.	.460	.258	.108	.591	.722	.681	.272	.298	.223	.479	.383	.851	.513	.686	.619	.101
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
IQ	Pearson Correlation	.243	-.283	1.000	-.402	-.435	.066	.640	.697*	.451	.520	-.078	-.150	.657	.000	.508	.615	.817**	.511
	Sig. (2-tailed)	.530	.460	.	.283	.242	.866	.063	.037	.223	.151	.854	.701	.055	1.000	.163	.078	.007	.160
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
BDI	Pearson Correlation	-.386	-.422	-.402	1.000	.651	.589	-.893**	-.452	-.301	-.260	.271	.367	-.563	.688*	-.673*	-.496	-.644	-.432
	Sig. (2-tailed)	.305	.258	.283	.	.058	.095	.001	.222	.431	.500	.517	.331	.115	.040	.047	.175	.061	.245
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
GHQ	Pearson Correlation	-.297	-.571	-.435	.651	1.000	.223	-.563	-.668*	-.470	-.458	.542	.698*	-.840**	.329	-.524	-.126	-.646	-.779*
	Sig. (2-tailed)	.438	.108	.242	.058	.	.564	.115	.049	.202	.215	.165	.037	.005	.387	.147	.747	.060	.013
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
BMI	Pearson Correlation	.111	-.208	.066	.589	.223	1.000	-.380	.115	.217	.261	-.283	.600	.030	.697*	-.046	-.187	-.051	-.007
	Sig. (2-tailed)	.776	.591	.866	.095	.564	.	.313	.768	.576	.498	.498	.088	.940	.037	.907	.631	.897	.987
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTDIGFO	Pearson Correlation	.159	.139	.640	-.893**	-.563	-.380	1.000	.702*	.290	.256	-.374	-.247	.674*	-.555	.768*	.620	.823**	.453
	Sig. (2-tailed)	.683	.722	.063	.001	.115	.313	.	.035	.450	.507	.362	.521	.047	.121	.016	.075	.006	.221
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTDIGBA	Pearson Correlation	-.055	.160	.697*	-.452	-.668*	.115	.702*	1.000	.393	.413	-.727*	-.271	.884**	-.088	.747*	.478	.913**	.778*
	Sig. (2-tailed)	.889	.681	.037	.222	.049	.768	.035	.	.296	.269	.041	.481	.002	.822	.021	.193	.001	.014
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTLETTE	Pearson Correlation	.545	.410	.451	-.301	-.470	.217	.290	.393	1.000	.974**	-.231	-.061	.658	.319	.411	.026	.536	.577
	Sig. (2-tailed)	.129	.272	.223	.431	.202	.576	.450	.296	.	.000	.581	.876	.054	.403	.272	.947	.137	.104
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTCAT	Pearson Correlation	.612	.391	.520	-.260	-.458	.261	.256	.413	.974**	1.000	-.215	-.006	.625	.408	.388	.130	.580	.657
	Sig. (2-tailed)	.080	.298	.151	.500	.215	.498	.507	.269	.000	.	.609	.987	.072	.276	.303	.738	.101	.055
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTERR	Pearson Correlation	.068	-.485	-.078	.271	.542	-.283	-.374	-.727*	-.231	-.215	1.000	-.011	-.704	.081	-.765*	-.245	-.551	-.606
	Sig. (2-tailed)	.872	.223	.854	.517	.165	.498	.362	.041	.581	.609	.	.980	.051	.848	.027	.558	.157	.111
	N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
TOTRT	Pearson Correlation	.216	-.272	-.150	.367	.698*	.600	-.247	-.271	-.061	-.006	-.011	1.000	-.453	.441	-.055	.155	-.201	-.368
	Sig. (2-tailed)	.577	.479	.701	.331	.037	.088	.521	.481	.876	.987	.980	.	.220	.234	.888	.691	.604	.330
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTHRECA	Pearson Correlation	.164	.332	.657	-.563	-.840**	.030	.674*	.884**	.658	.625	-.704	-.453	1.000	-.171	.766*	.303	.853**	.780*
	Sig. (2-tailed)	.674	.383	.055	.115	.005	.940	.047	.002	.054	.072	.051	.220	.	.659	.016	.428	.004	.013
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTHRECO	Pearson Correlation	.061	-.073	.000	.688*	.329	.697*	-.555	-.088	.319	.408	.081	.441	-.171	1.000	-.426	-.324	-.134	.108
	Sig. (2-tailed)	.875	.851	1.000	.040	.387	.037	.121	.822	.403	.276	.848	.234	.659	.	.253	.394	.730	.782
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTDOOR	Pearson Correlation	.221	.252	.508	-.673*	-.524	-.046	.768*	.747*	.411	.388	-.765*	-.055	.766*	-.426	1.000	.686*	.810**	.551
	Sig. (2-tailed)	.568	.513	.163	.047	.147	.907	.016	.021	.272	.303	.027	.888	.016	.253	.	.041	.008	.124
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTNAME	Pearson Correlation	.220	-.158	.615	-.496	-.126	-.187	.620	.478	.026	.130	-.245	.155	.303	-.324	.686*	1.000	.678*	.351
	Sig. (2-tailed)	.569	.686	.078	.175	.747	.631	.075	.193	.947	.738	.558	.691	.428	.394	.041	.	.045	.354
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
TOTSPEED	Pearson Correlation	.220	.193	.817**	-.644	-.646	-.051	.823**	.913**	.536	.580	-.551	-.201	.853**	-.134	.810**	.678*	1.000	.801**
	Sig. (2-tailed)	.569	.619	.007	.061	.060	.897	.006	.001	.137	.101	.157	.604	.004	.730	.008	.045	.	.009
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
LOGSPOT	Pearson Correlation	.282	.581	.511	-.432	-.779*	-.007	.453	.778*	.577	.657	-.606	-.368	.780*	.108	.551	.351	.801**	1.000
	Sig. (2-tailed)	.463	.101	.160	.245	.013	.987	.221	.014	.104	.055	.111	.330	.013	.782	.124	.354	.009	.
	N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Correlations

			AGE	TREATDUR	IQ	BDI	GHQ	BMI	TOTDIGFO	TOTDIGBA	TOTLETTE	TOTCAT	TOTERR	TOTRT	TOTHRECA	TOTHRECO	TOTDOOR	TOTNAME	TOTSPEED	LOGSPOT
Spearman's rho	AGE	Correlation Coefficient	1.000	.256	.377	-.557	-.378	.033	.265	.046	.399	.695*	.012	.393	.197	.092	.201	.264	.239	.430
		Sig. (2-tailed)	.	.506	.318	.119	.316	.932	.491	.906	.287	.038	.977	.295	.611	.815	.604	.493	.535	.248
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TREATDUR	Correlation Coefficient	.256	1.000	-.226	-.460	-.714*	.017	.210	.340	.471	.326	-.325	-.360	.370	.000	.276	-.213	.349	.637
		Sig. (2-tailed)	.506	.	.559	.213	.031	.966	.587	.370	.201	.391	.432	.342	.327	1.000	.472	.583	.358	.065
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	IQ	Correlation Coefficient	.377	-.226	1.000	-.345	-.184	.200	.661	.594	.226	.483	.012	-.083	.527	.091	.333	.509	.661	.378
		Sig. (2-tailed)	.318	.559	.	.364	.635	.606	.053	.092	.559	.187	.976	.831	.145	.815	.381	.162	.053	.316
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	BDI	Correlation Coefficient	-.557	-.460	-.345	1.000	.650	.378	-.835**	-.477	-.481	-.445	.248	.101	-.464	.598	-.571	-.393	-.519	-.470
		Sig. (2-tailed)	.119	.213	.364	.	.058	.316	.005	.194	.190	.230	.553	.796	.208	.089	.108	.295	.152	.201
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	GHQ	Correlation Coefficient	-.378	-.714*	-.184	.650	1.000	-.017	-.496	-.685*	-.689*	-.711*	.657	.510	-.798**	.229	-.678*	.102	-.601	-.844**
		Sig. (2-tailed)	.316	.031	.635	.058	.	.966	.175	.042	.040	.032	.077	.160	.010	.553	.045	.794	.087	.004
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	BMI	Correlation Coefficient	.033	.017	.200	.378	-.017	1.000	-.201	.276	.335	.267	-.515	.233	.184	.822**	.100	-.220	.259	.176
		Sig. (2-tailed)	.932	.966	.606	.316	.966	.	.604	.472	.379	.488	.192	.546	.635	.007	.798	.569	.500	.650
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTDIGFO	Correlation Coefficient	.265	.210	.661	-.835**	-.496	-.201	1.000	.739*	.445	.377	-.240	-.268	.618	-.504	.661	.570	.773*	.460
		Sig. (2-tailed)	.491	.587	.053	.005	.175	.604	.	.023	.230	.318	.568	.486	.076	.166	.053	.109	.015	.213
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTDIGBA	Correlation Coefficient	.046	.340	.594	-.477	-.685*	.276	.739*	1.000	.546	.552	-.651	-.485	.824**	-.092	.770*	.306	.912**	.747*
		Sig. (2-tailed)	.906	.370	.092	.194	.042	.472	.023	.	.128	.123	.081	.185	.006	.815	.015	.423	.001	.021
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTLETTE	Correlation Coefficient	.399	.471	.226	-.481	-.689*	.335	.445	.546	1.000	.745*	-.707	-.184	.786*	-.046	.795*	-.009	.672*	.646
		Sig. (2-tailed)	.287	.201	.559	.190	.040	.379	.230	.128	.	.021	.050	.635	.012	.907	.010	.983	.047	.060
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTCAT	Correlation Coefficient	.695*	.326	.483	-.445	-.711*	.267	.377	.552	.745*	1.000	-.575	-.100	.736*	.091	.700*	.237	.678*	.649**
		Sig. (2-tailed)	.038	.391	.187	.230	.032	.488	.318	.123	.021	.	.136	.798	.024	.815	.036	.539	.045	.004
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTERR	Correlation Coefficient	.012	-.325	.012	.248	.657	-.515	-.240	-.651	-.707	-.575	1.000	.096	-.645	.000	-.874**	-.172	-.560	-.600
		Sig. (2-tailed)	.977	.432	.978	.553	.077	.192	.568	.081	.050	.136	.	.821	.084	1.000	.005	.684	.149	.116
		N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	TOTRT	Correlation Coefficient	.393	-.360	-.083	.101	.510	.233	-.268	-.485	-.184	-.100	.096	1.000	-.628	.274	-.267	.254	-.360	-.445
		Sig. (2-tailed)	.295	.342	.831	.796	.160	.546	.486	.185	.635	.798	.821	.	.070	.476	.488	.509	.342	.230
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTHRECA	Correlation Coefficient	.197	.370	.527	-.464	-.798**	.184	.618	.824**	.786*	.736*	-.645	-.628	1.000	-.138	.787*	.043	.824**	.793*
		Sig. (2-tailed)	.611	.327	.145	.208	.010	.635	.076	.006	.012	.024	.084	.070	.	.724	.012	.913	.006	.011
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTHRECO	Correlation Coefficient	.092	.000	.091	.598	.229	.822**	-.504	-.092	-.046	.091	.000	.274	-.138	1.000	-.365	-.279	-.046	.046
		Sig. (2-tailed)	.815	1.000	.815	.089	.553	.007	.166	.815	.907	.815	1.000	.476	.724	.	.334	.468	.907	.906
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTDOOR	Correlation Coefficient	.201	.276	.333	-.571	-.678*	.100	.661	.770*	.795*	.700*	-.874**	-.267	.787*	-.365	1.000	.390	.812**	.689*
		Sig. (2-tailed)	.604	.472	.381	.108	.045	.798	.053	.015	.010	.036	.005	.488	.012	.334	.	.300	.008	.040
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTNAME	Correlation Coefficient	.264	-.213	.509	-.393	.102	-.220	.570	.306	-.009	.237	-.172	.254	.043	-.279	.390	1.000	.494	.205
		Sig. (2-tailed)	.493	.583	.162	.295	.794	.569	.109	.423	.983	.539	.684	.509	.913	.468	.300	.	.177	.596
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	TOTSPEED	Correlation Coefficient	.239	.349	.661	-.519	-.601	.259	.773*	.912**	.672*	.678*	-.560	-.360	.824**	-.046	.812**	.494	1.000	.797*
		Sig. (2-tailed)	.535	.358	.053	.152	.087	.500	.015	.001	.047	.045	.149	.342	.006	.907	.008	.177	.	.010
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9
	LOGSPOT	Correlation Coefficient	.430	.637	.378	-.470	-.844**	.176	.460	.747*	.646	.649**	-.600	-.445	.793*	.046	.689*	.205	.797*	1.000
		Sig. (2-tailed)	.248	.065	.316	.201	.004	.650	.213	.021	.060	.004	.116	.230	.011	.906	.040	.596	.010	.
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9

* Correlation is significant at the .05 level (2-tailed).

** Correlation is significant at the .01 level (2-tailed).

Correlations

			LOGSPOT	TOTDIGFO	TOTDIGBA	TOTLETTE	TOTCAT	TOTHRECA	TOTHRECO	TOTDOOR	TOTNAME	TOTSPEED	TOTERR	TOTRT	TOTCAF	TOTGLUC
Spearman's rho	LOGSPOT	Correlation Coefficient	1.000	.460	.747*	.646	.849**	.793*	.046	.689*	.205	.797*	-.600	-.445	-.202	.096
		Sig. (2-tailed)	.	.213	.021	.060	.004	.011	.906	.040	.596	.010	.116	.230	.603	.821
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTDIGFO	Correlation Coefficient	.460	1.000	.739*	.445	.377	.618	-.504	.661	.570	.773*	-.240	-.268	-.226	.371
		Sig. (2-tailed)	.213	.	.023	.230	.318	.076	.166	.053	.109	.015	.568	.486	.559	.365
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTDIGBA	Correlation Coefficient	.747*	.739*	1.000	.546	.552	.824**	-.092	.770*	.306	.912**	-.651	-.485	-.059	.333
		Sig. (2-tailed)	.021	.023	.	.128	.123	.006	.815	.015	.423	.001	.081	.185	.881	.420
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTLETTE	Correlation Coefficient	.646	.445	.546	1.000	.745*	.786*	-.046	.795*	-.009	.672*	-.707	-.184	-.494	.036
		Sig. (2-tailed)	.060	.230	.128	.	.021	.012	.907	.010	.983	.047	.050	.635	.177	.933
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTCAT	Correlation Coefficient	.849**	.377	.552	.745*	1.000	.736*	.091	.700*	.237	.678*	-.575	-.100	-.150	.238
		Sig. (2-tailed)	.004	.318	.123	.021	.	.024	.815	.036	.539	.045	.136	.798	.700	.570
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTHRECA	Correlation Coefficient	.793*	.618	.824**	.786*	.736*	1.000	-.138	.787*	.043	.824**	-.645	-.628	-.393	.359
		Sig. (2-tailed)	.011	.076	.006	.012	.024	.	.724	.012	.913	.006	.084	.070	.295	.382
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTHRECO	Correlation Coefficient	.046	-.504	-.092	-.046	.091	-.138	1.000	-.365	-.279	-.046	.000	.274	.183	-.507
		Sig. (2-tailed)	.906	.166	.815	.907	.815	.724	.	.334	.468	.907	1.000	.476	.638	.200
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTDOOR	Correlation Coefficient	.689*	.661	.770*	.795*	.700*	.787*	-.365	1.000	.390	.812**	-.874**	-.267	-.283	.357
		Sig. (2-tailed)	.040	.053	.015	.010	.036	.012	.334	.	.300	.008	.005	.488	.460	.385
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTNAME	Correlation Coefficient	.205	.570	.306	-.009	.237	.043	-.279	.390	1.000	.494	-.172	.254	-.051	.024
		Sig. (2-tailed)	.596	.109	.423	.983	.539	.913	.468	.300	.	.177	.684	.509	.897	.954
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTSPEED	Correlation Coefficient	.797*	.773*	.912**	.672*	.678*	.824**	-.046	.812**	.494	1.000	-.560	-.360	-.335	.119
		Sig. (2-tailed)	.010	.015	.001	.047	.045	.006	.907	.008	.177	.	.149	.342	.379	.779
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTERR	Correlation Coefficient	-.600	-.240	-.651	-.707	-.575	-.645	.000	-.874**	-.172	-.560	1.000	.096	-.108	-.252
		Sig. (2-tailed)	.116	.568	.081	.050	.136	.084	1.000	.005	.684	.149	.	.821	.799	.585
		N	8	8	8	8	8	8	8	8	8	8	8	8	8	7
	TOTRT	Correlation Coefficient	-.445	-.268	-.485	-.184	-.100	-.628	.274	-.267	.254	-.360	.096	1.000	.400	-.024
		Sig. (2-tailed)	.230	.486	.185	.635	.798	.070	.476	.488	.509	.342	.821	.	.286	.955
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTCAF	Correlation Coefficient	-.202	-.226	-.059	-.494	-.150	-.393	.183	-.283	-.051	-.335	-.108	.400	1.000	.548
		Sig. (2-tailed)	.603	.559	.881	.177	.700	.295	.638	.460	.897	.379	.799	.286	.	.160
		N	9	9	9	9	9	9	9	9	9	9	8	9	9	8
	TOTGLUC	Correlation Coefficient	.096	.371	.333	.036	.238	.359	-.507	.357	.024	.119	-.252	-.024	.548	1.000
		Sig. (2-tailed)	.821	.365	.420	.933	.570	.382	.200	.385	.954	.779	.585	.955	.160	.
		N	8	8	8	8	8	8	8	8	8	8	7	8	8	8

* Correlation is significant at the .05 level (2-tailed).

** Correlation is significant at the .01 level (2-tailed).

Correlations

			TREATDUR	LOGSPOT	TOTDIGFO	TOTDIGBA	TOTLETTE	TOTCAT	TOTHRECA	TOTHRECO	TOTDOOR	TOTNAME	TOTSPEED	TOTERR	TOTRT
Spearman's rho	TREATDUR	Correlation Coefficient	1.000	.500	-1.000**	-.500	.500	.500	.500	.000	1.000**	-.866	-.500	-1.000**	-.500
		Sig. (2-tailed)	.	.667	.000	.667	.667	.667	.667	1.000	.	.333	.667	.000	.667
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	LOGSPOT	Correlation Coefficient	.500	1.000	-.500	.500	1.000**	1.000**	1.000**	.866	.500	-.866	.500	-.500	-1.000**
		Sig. (2-tailed)	.667	.	.667	.667	.	.	.	.333	.667	.333	.667	.667	.000
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTDIGFO	Correlation Coefficient	-1.000**	-.500	1.000	.500	-.500	-.500	-.500	.000	-1.000**	.866	.500	1.000**	.500
		Sig. (2-tailed)	.000	.667	.	.667	.667	.667	.667	1.000	.000	.333	.667	.	.667
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTDIGBA	Correlation Coefficient	-.500	.500	.500	1.000	.500	.500	.500	.866	-.500	.000	1.000**	.500	-.500
		Sig. (2-tailed)	.667	.667	.667	.	.667	.667	.667	.333	.667	1.000	.	.667	.667
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTLETTE	Correlation Coefficient	.500	1.000**	-.500	.500	1.000	1.000**	1.000**	.866	.500	-.866	.500	-.500	-1.000**
		Sig. (2-tailed)	.667	.	.667	.667	.	.	.	.333	.667	.333	.667	.667	.000
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTCAT	Correlation Coefficient	.500	1.000**	-.500	.500	1.000**	1.000	1.000**	.866	.500	-.866	.500	-.500	-1.000**
		Sig. (2-tailed)	.667	.	.667	.667	.	.	.	.333	.667	.333	.667	.667	.000
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTHRECA	Correlation Coefficient	.500	1.000**	-.500	.500	1.000**	1.000**	1.000	.866	.500	-.866	.500	-.500	-1.000**
		Sig. (2-tailed)	.667	.	.667	.667	.	.	.	.333	.667	.333	.667	.667	.000
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTHRECO	Correlation Coefficient	.000	.866	.000	.866	.866	.866	.866	1.000	.000	-.500	.866	.000	-.866
		Sig. (2-tailed)	1.000	.333	1.000	.333	.333	.333	.333	.	1.000	.667	.333	1.000	.333
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTDOOR	Correlation Coefficient	1.000**	.500	-1.000**	-.500	.500	.500	.500	.000	1.000	-.866	-.500	-1.000**	-.500
		Sig. (2-tailed)	.	.667	.000	.667	.667	.667	.667	1.000	.	.333	.667	.000	.667
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTNAME	Correlation Coefficient	-.866	-.866	.866	.000	-.866	-.866	-.866	-.500	-.866	1.000	.000	.866	.866
		Sig. (2-tailed)	.333	.333	.333	1.000	.333	.333	.333	.667	.333	.	1.000	.333	.333
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTSPEED	Correlation Coefficient	-.500	.500	.500	1.000**	.500	.500	.500	.866	-.500	.000	1.000	.500	-.500
		Sig. (2-tailed)	.667	.667	.667	.	.667	.667	.667	.333	.667	1.000	.	.667	.667
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTERR	Correlation Coefficient	-1.000**	-.500	1.000**	.500	-.500	-.500	-.500	.000	-1.000**	.866	.500	1.000	.500
		Sig. (2-tailed)	.000	.667	.	.667	.667	.667	.667	1.000	.000	.333	.667	.	.667
		N	3	3	3	3	3	3	3	3	3	3	3	3	3
	TOTRT	Correlation Coefficient	-.500	-1.000**	.500	-.500	-1.000**	-1.000**	-1.000**	-.866	-.500	.866	-.500	.500	1.000
		Sig. (2-tailed)	.667	.000	.667	.667	.000	.000	.000	.333	.667	.333	.667	.667	.
		N	3	3	3	3	3	3	3	3	3	3	3	3	3

**. Correlation is significant at the .01 level (2-tailed).

Correlations

			TREATDUR	LOGSPOT	TOTDIGFO	TOTDIGBA	TOTLETTE	TOTCAT	TOTHRECA	TOTHRECO	TOTDOOR	TOTNAME	TOTSPEED	TOTERR	TOTRT
Spearman's rho	TREATDUR	Correlation Coefficient	1.000	.382	.500	.397	.088	-.464	-.059	-.210	-.087	-.116	.290	.051	-.377
		Sig. (2-tailed)	.	.454	.313	.436	.868	.354	.912	.690	.870	.827	.577	.935	.461
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	LOGSPOT	Correlation Coefficient	.382	1.000	.544	.971**	.456	.493	.647	-.105	.551	.348	.812*	-.821	-.551
		Sig. (2-tailed)	.454	.	.264	.001	.364	.321	.165	.843	.257	.499	.050	.089	.257
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTDIGFO	Correlation Coefficient	.500	.544	1.000	.529	.824*	.290	.544	-.735	.754	.522	.754	-.600	-.232
		Sig. (2-tailed)	.313	.264	.	.280	.044	.577	.264	.096	.084	.288	.084	.285	.658
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTDIGBA	Correlation Coefficient	.397	.971**	.529	1.000	.529	.551	.618	.000	.580	.290	.812*	-.872	-.406
		Sig. (2-tailed)	.436	.001	.280	.	.280	.257	.191	1.000	.228	.577	.050	.054	.425
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTLETTE	Correlation Coefficient	.088	.456	.824*	.529	1.000	.696	.691	-.525	.928**	.464	.696	-.800	.058
		Sig. (2-tailed)	.868	.364	.044	.280	.	.125	.128	.285	.008	.354	.125	.104	.913
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTCAT	Correlation Coefficient	-.464	.493	.290	.551	.696	1.000	.667	.000	.829*	.486	.600	-.900*	.143
		Sig. (2-tailed)	.354	.321	.577	.257	.125	.	.148	1.000	.042	.329	.208	.037	.787
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTHRECA	Correlation Coefficient	-.059	.647	.544	.618	.691	.667	1.000	-.525	.667	.116	.464	-.821	-.551
		Sig. (2-tailed)	.912	.165	.264	.191	.128	.148	.	.285	.148	.827	.354	.089	.257
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTHRECO	Correlation Coefficient	-.210	-.105	-.735	.000	-.525	.000	-.525	1.000	-.414	-.207	-.207	.000	.414
		Sig. (2-tailed)	.690	.843	.096	1.000	.285	1.000	.285	.	.414	.694	.694	1.000	.414
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTDOOR	Correlation Coefficient	-.087	.551	.754	.580	.928**	.829*	.667	-.414	1.000	.714	.829*	-.900*	.086
		Sig. (2-tailed)	.870	.257	.084	.228	.008	.042	.148	.414	.	.111	.042	.037	.872
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTNAME	Correlation Coefficient	-.116	.348	.522	.290	.464	.486	.116	-.207	.714	1.000	.771	-.700	.257
		Sig. (2-tailed)	.827	.499	.288	.577	.354	.329	.827	.694	.111	.	.072	.188	.623
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTSPEED	Correlation Coefficient	.290	.812*	.754	.812*	.696	.600	.464	-.207	.829*	.771	1.000	-.900*	-.086
		Sig. (2-tailed)	.577	.050	.084	.050	.125	.208	.354	.694	.042	.072	.	.037	.872
		N	6	6	6	6	6	6	6	6	6	6	6	5	6
	TOTERR	Correlation Coefficient	.051	-.821	-.600	-.872	-.800	-.900*	-.821	.000	-.900*	-.700	-.900*	1.000	.000
		Sig. (2-tailed)	.935	.089	.285	054	.104	.037	.089	1.000	.037	.188	.037	.	1.000
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
	TOTRT	Correlation Coefficient	-.377	-.551	-.232	-.406	.058	.143	-.551	.414	.086	.257	-.086	.000	1.000
		Sig. (2-tailed)	.461	.257	.658	.425	.913	.787	.257	.414	.872	.623	.872	1.000	.
		N	6	6	6	6	6	6	6	6	6	6	6	5	6

**. Correlation is significant at the .01 level (2-tailed)

*. Correlation is significant at the .05 level (2-tailed)

Correlations

			TREATDUR	LOGSPOT	TOTDIGFO	TOTDIGBA	TOTLETTE	TOTCAT	TOTHRECA	TOTHRECO	TOTDOOR	TOTNAME	TOTSPEED	TOTERR	TOTRT
Spearman's rho	TREATDUR	Correlation Coefficient	1.000	.637	.210	.340	.471	.326	.370	.000	.276	-.213	.349	-.325	-.360
		Sig. (2-tailed)	.	.065	.587	.370	.201	.391	.327	1.000	.472	.583	.358	.432	.342
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	LOGSPOT	Correlation Coefficient	.637	1.000	.460	.747*	.646	.849**	.793*	.046	.689*	.205	.797*	-.600	-.445
		Sig. (2-tailed)	.065	.	.213	.021	.060	.004	.011	.906	.040	.596	.010	.116	.230
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTDIGFO	Correlation Coefficient	.210	.460	1.000	.739*	.445	.377	.618	-.504	.661	.570	.773*	-.240	-.268
		Sig. (2-tailed)	.587	.213	.	.023	.230	.318	.076	.166	.053	.109	.015	.568	.486
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTDIGBA	Correlation Coefficient	.340	.747*	.739*	1.000	.546	.552	.824**	-.092	.770*	.306	.912**	-.651	-.485
		Sig. (2-tailed)	.370	.021	.023	.	.128	.123	.006	.815	.015	.423	.001	.081	.185
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTLETTE	Correlation Coefficient	.471	.646	.445	.546	1.000	.745*	.786*	-.046	.795*	-.009	.672*	-.707	-.184
		Sig. (2-tailed)	.201	.060	.230	.128	.	.021	.012	.907	.010	.983	.047	.050	.635
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTCAT	Correlation Coefficient	.326	.849**	.377	.552	.745*	1.000	.736*	.091	.700*	.237	.678*	-.575	-.100
		Sig. (2-tailed)	.391	.004	.318	.123	.021	.	.024	.815	.036	.539	.045	.136	.798
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTHRECA	Correlation Coefficient	.370	.793*	.618	.824**	.786*	.736*	1.000	-.138	.787*	.043	.824**	-.645	-.628
		Sig. (2-tailed)	.327	.011	.076	.006	.012	.024	.	.724	.012	.913	.006	.084	.070
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTHRECO	Correlation Coefficient	.000	.046	-.504	-.092	-.046	.091	-.138	1.000	-.365	-.279	-.046	.000	.274
		Sig. (2-tailed)	1.000	.906	.166	.815	.907	.815	.724	.	.334	.468	.907	1.000	.476
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTDOOR	Correlation Coefficient	.276	.689*	.661	.770*	.795*	.700*	.787*	-.365	1.000	.390	.812**	-.874**	-.267
		Sig. (2-tailed)	.472	.040	.053	.015	.010	.036	.012	.334	.	.300	.008	.005	.488
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTNAME	Correlation Coefficient	-.213	.205	.570	.306	-.009	.237	.043	-.279	.390	1.000	.494	-.172	.254
		Sig. (2-tailed)	.583	.596	.109	.423	.983	.539	.913	.468	.300	.	.177	.684	.509
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTSPEED	Correlation Coefficient	.349	.797*	.773*	.912**	.672*	.678*	.824**	-.046	.812**	.494	1.000	-.560	-.360
		Sig. (2-tailed)	.358	.010	.015	.001	.047	.045	.006	.907	.008	.177	.	.149	.342
		N	9	9	9	9	9	9	9	9	9	9	9	8	9
	TOTERR	Correlation Coefficient	-.325	-.600	-.240	-.651	-.707	-.575	-.645	.000	-.874**	-.172	-.560	1.000	.096
		Sig. (2-tailed)	.432	.116	.568	.081	.050	.136	.084	1.000	.005	.684	.149	.	.821
		N	8	8	8	8	8	8	8	8	8	8	8	8	8
	TOTRT	Correlation Coefficient	-.360	-.445	-.268	-.485	-.184	-.100	-.628	.274	-.267	.254	-.360	.096	1.000
		Sig. (2-tailed)	.342	.230	.486	.185	.635	.798	.070	.476	.488	.509	.342	.821	.
		N	9	9	9	9	9	9	9	9	9	9	9	8	9

* Correlation is significant at the .05 level (2-tailed)

** Correlation is significant at the .01 level (2-tailed)